PR

Phytopathology

An International Journal

THE UNIVERSITY OF MICHIGAN
FEB 16 1960
SCIENCE LIBRARY

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

VOLUME 50 JANUARY, 1960 NUMBER 1

Influence of soil temperature on seedling blight of smooth bromegrass
IVAN J. THOMASON AND J. G. DICKSON 1

A strain of cucumber virus 1 infectious to blight-resistant spinach
S. P. DOOLITTLE AND R. E. WEBB 7

Hydrolytic and respiratory enzymes of species of Ditylenchus and Pratylenchus L. R. KRUSBERG 9

Effect of growing crops and crop residues on soil fungi and seedling blights
LANSING E. WILLIAMS AND A. F. SCHMITTHENNER 22

Influence of boron nutrition of Nicotiana tabacum on the multiplication of tobacco mosaic virus
ROBERT J. SHEPHERD AND GLENN S. POUND 26

Similar metabolic alterations induced in sweet potato by poisonous chemicals and by Ceratostomella fimbriata I. URITANI, M. URITANI, AND H. YAMADA 30

The antigenic characteristics and the relationship among strains of alfalfa mosaic virus
J. B. BANCROFT, ELLEN L. MOORHEAD, J. TUITE, AND H. P. LIU 34

Survival of physiologic races of Puccinia graminis var. tritici on wheat near barberry bushes
C. W. ROANE, E. C. STAKMAN, W. Q. LOEGERING, D. M. STEWART, AND W. M. WATSON 40

Inheritance of resistance to Fusarium wilt in Upland and Sea Island cottons as complicated by nematodes under field conditions A. L. SMITH AND J. B. DICK 44

Inoculation of slash pine seedlings with Cronartium fusiforme F. F. JEWELL 48

Differential longevity of teliospores of pathogenic races of Tilletia caries and T. foetida
E. L. KENDRICK AND C. S. HOLTON 51

A new source of resistance to spinach blight
R. E. WEBB, BRUCE A. PERRY, HENRY A. JONES, AND D. M. MCLEAN 54

Physiology of Fusarium solani f. phaseoli in relation to saprophytic survival in soil OTIS C. MALOY 56

The effect of environment on germination of sporidia in Cronartium ribicola ROBERT V. BEGA 61

A precision spray technique for evaluating oils for Sigatoka disease control on individual banana leaves in the field L. CALPOUZOS, W. A. BRUN, T. THEIS, AND C. COLBERG 69

Inheritance of resistance to potato virus Y derived from two strains of Capsicum annuum
A. A. COOK AND C. W. ANDERSON 73

Onion blast or leaf spotting caused by species of Botrytis R. H. SEGALL AND A. G. NEWHALL 76

Report and abstracts of the 1959 annual meeting of the Pacific Division of the American Phytopathological Society 82

Phytopathological notes:

An enrichment method for isolating actinomycetes that produce diffusible antifungal antibiotics
PETER H. TSAO, CURT LEBEN, AND G. W. KEITT 88

On the infectivity dilution curve of plant viruses ALBERT SIEGEL 89

Book listings 90

News

Office of Publication—Monumental Printing Company, Baltimore, Md.

Subscription, $10.00 per year Entered as second class matter at the post office at Baltimore, Md.

Plant Pathology

An Advanced Treatise

Edited by J. G. HORSFALL *and* A. E. DIMOND

The Connecticut Agricultural Experiment Station, New Haven

10% discount on orders for the complete set

This treatise presents a synthesis of the principles of plant pathology, giving primary consideration to the plant rather than to the pathogen. Emphasis is placed upon the obstruction of normal pathways in diseased plants, the mechanisms by which pathogens bring about dysfunction, and the reaction of the host plant to the pathogen.

Volume 1

The Diseased Plant

October 1959, 674 pp., illus., $22.00

Prologue: The Diseased Plant
By J. G. HORSFALL *and* A. E. DIMOND

Scope and Contributions of Plant Pathology
By J. G. TEN HOUTEN

History of Plant Pathology
By G. W. KEITT

How Sick is the Plant?
By K. STARR CHESTER

Tissue Is Disintegrated
By AKHTAR HUSAIN *and* ARTHUR KELMAN

Growth Is Affected
By ARMIN C. BRAUN

Reproduction Is Affected
By ANTONIO CICCARONE

The Host Is Starved
By C. SEMPIO

Water Is Deficient
By D. SUBRAMANIAN *and* L. SARASWATHI-DEVI

Alteration of the Respiratory Pattern in Infected Plants
By IKUZO URITANI *and* TAKASHI AKAZAWA

Histology of Defense in Plants
By S. AKAI

Physiology and Biochemistry of Defense
By PAUL J. ALLEN

Hypersensitivity
By K. O. MÜLLER

Predisposition
By C. E. YARWOOD

Therapy
By F. L. HOWARD *and* J. G. HORSFALL

AUTHOR INDEX—SUBJECT INDEX

Volume 2

The Pathogen

January 1960, about 700 pp., illus., approx. $22.00

Volume 3

The Diseased Population—Epidemics and Control

Ready early 1960, in preparation

Complete contents of all volumes available upon request

(Listing of PLANT PHYSIOLOGY, in 6 volumes, will appear in the February issue.)

ACADEMIC PRESS, ***New York and London***

111 Fifth Avenue, New York 3, New York

17 Old Queen Street, London, S.W. 1

PHYTOPATHOLOGY—An International Journal—Official Organ of the American Phytopathological Society
FOUNDED 1908—INCORPORATED 1915

EDITORS

E. E. Wilson, *Editor-in-Chief*, Department of Plant Pathology, University of California, Davis; **Kenneth F. Baker,** University of California, Los Angeles; **George W. Bruehl,** Washington State University, Pullman; **E. C. Calavan,** University of California, Riverside; **J. M. Daly,** University of Nebraska, Lincoln; **Stephen Diachun,** University of Kentucky, Lexington; **Charles L. Fergus,** Pennsylvania State University, University Park; **Robert W. Fulton,** University of Wisconsin, Madison; **Max W. Gardner,** University of California, Berkeley; **David Gottlieb,** University of Illinois, Urbana; **James G. Horsfall,** Connecticut Agricultural Experiment Station, New Haven; **William F. Mai,** Cornell University, Ithaca, New York; **John H. Owen,** University of Georgia, Athens; **Marr D. Simons,** Iowa State College, Ames; **Hugh D. Sisler,** University of Maryland, College Park; **Edwin B. Williams,** Purdue University, Lafayette, Indiana.

BUSINESS MANAGEMENT

Business Manager, *A. W. Dimock*, 32nd St. and Elm Ave., Baltimore, Maryland, or R.D. 2, Ithaca, N. Y. **Advertising Manager,** *D. A. Roberts*, Plant Pathology Department, University of Florida, Gainesville.

THE A.P.S. COUNCIL

President, *William C. Snyder*, University of California, Berkeley; **Vice-President,** *William B. Hewitt*, University of California, Davis; **Secretary,** *George A. Zentmyer*, University of California, Riverside; **Treasurer,** *A. W. Dimock*, R.D. 2, Ithaca, N. Y.; **Editor-in-Chief,** *E. E. Wilson*, University of California, Davis; **President-Elect,** *S. E. A. McCallan*, Boyce Thompson Institute, Yonkers 3, New York; **Retiring President,** *Glenn S. Pound*, University of Wisconsin, Madison 6.

DIVISIONS OF THE SOCIETY

Northeastern: *Pres.*, J. E. Livingston, Pennsylvania State University, University Park; *Vice-Pres.*, D. H. Marsden, Eastern St. Farmers' Exchange, West Springfield, Massachusetts; *Sec.-Treas.*, R. H. Daines, College of Agriculture, New Brunswick, New Jersey. **North Central:** *Pres.*, D. F. Millikan, University of Missouri, Columbia; *Vice-Pres.*, C. M. Nagel, South Dakota State College, Brookings; *Sec.-Treas.*, E. W. Hanson, University of Wisconsin, Madison 6. **Pacific:** *Pres.*, K. F. Baker, University of California, Los Angeles; *Vice-Pres.*, J. D. Menzies, Irrigation Experiment Station, Prosser, Washington; *Sec.-Treas.*, William Kreutzer, Shell Agricultural Laboratory, Modesto, California. **Potomac:** *Pres.*, W. D. McClellan, Agricultural Research Service, Washington, D. C.; *Vice-Pres.*, A. B. Groves, Winchester Fruit Research Laboratory, Winchester, Virginia; *Sec.-Treas.*, John G. Moseman, Plant Industry Station, Beltsville, Maryland. **Southern:** *Pres.*, E. M. Cralley, University of Arkansas, Fayetteville; *Vice-Pres.*, W. J. Martin, Louisiana State University, Baton Rouge; *Sec.-Treas.*, J. P. Fulton, University of Arkansas, Fayetteville.

SUSTAINING ASSOCIATES

American Tobacco Company, Inc., Research Laboratory, 400 Petersburg Turnpike, Richmond 24, Virginia; **The Dow Chemical Company,** Midland, Michigan; **E. I. du Pont de Nemour & Company, Inc.,** Wilmington 98, Delaware; **Ferry-Morse Seed Company,** P. O. Box 778, Detroit 31, Michigan; **Freeport Sulphur Company,** New Orleans 5, Louisiana; **H. J. Heinz Company,** Pittsburgh 30, Pennsylvania; **Mathieson Chemical Corporation,** Mathieson Building, Baltimore, Maryland; **Monsanto Chemical Company,** 800 N. Lindberg Boulevard, St. Louis 66, Missouri; **Rohm & Haas Company,** Washington Square, Philadelphia 5, Pennsylvania; **Shell Chemical Corporation,** Agricultural Chemicals Sales Division, 460 Park Avenue, New York 22, N. Y.; **Tennessee Corporation,** 619-27 Grand Building, Atlanta 1, Georgia; **Yoder Brothers, Inc.,** Barberton, Ohio.

Advertisements.—Rates may be secured from the Advertising Manager. No endorsement of any claims in advertisements is assumed by this Journal or by The American Phytopathological Society.

Subscriptions and back numbers.—Write to the Business Manager. **Subscription price.**—$10.00 per year, domestic and foreign. Single current numbers (within 60 days of publication), $1.00. The journal has been issued monthly begininng with Vol. 8. **Separate numbers** from back volumes, $1.00. **Complete unbound volumes:** 1–7, $6.00 when available; 8–41, $10.00 when available. **Thirty-year index** (vols. 1–30, 1911–40), bound: $7.50, domestic and foreign. **Ten-year index** (vols. 31–40, 1941–50), paper cover: $3.50. **Lost numbers.**—Claims for copies (domestic subscriptions) lost in the mails must be received within 60 days of date of issue; otherwise replacement charge is the usual $1.00. Deliveries overseas are not guaranteed; foreign mailings are made at the subscriber's risk. **Separates.**—No gratis copies supplied. Quotation is sent with proof.

To speed your manuscript—in review, editing, and publication—follow correct style, described below by example and explanation. Articles by Price and Riker (1, 2) provide additional details.

WRITING FOR PHYTOPATHOLOGY

John Doe

Professor, Department of Botany, University of Blank.

A report on *Phytopathology* style beginning in 1960.

Note that titlenotes and authornotes are no longer treated as footnotes.

Accepted for publication

SUMMARY

Center this heading; don't underscore. May be written for use also as abstract in Biological Abstracts. Keep it *close* to 3% of paper, conveying contents when read *independently*. Give facts; don't just refer to them with "is described," etc. Type line below:

Introduction.—State problem briefly and reason for presenting findings.

Review of literature.—If one is needed, it should follow introduction and precede:

Materials and methods.—Describe general materials and methods *once*. Those for a particular experiment should be deferred to the appropriate place.

Results.—Present in logical rather than chronological order, usually subdivided. Reader should get clear picture of results, but don't interpret the obvious.

Discussion.—This section is *not* for a summary (which begins the paper). So summarize only the minimum for discussing implications of your findings or relating them to major problems in the field. Close with institution where work was done.—Department of Botany, University of Blank.

Don't start a new page; follow immediately with:

LITERATURE CITED

1. Price, W. C. 1954. Preparing manuscripts for Phytopathology. Phytopathology 44:667-674.

2. Riker, A. J. 1946. The preparation of manuscripts for Phytopathology. Phytopathology 36:953-977.

Even a single citation is to be under the above heading, not in a footnote. Don't underline *anything*. For male authors, use initials only. List alphabetically. Order by dates is used only if authors are *identical*, e.g., *Smith and Jones* always follows *Smith* alone. Abbreviate in the style of *Chemical Abstracts* "List of Periodicals"; most common are: Abstr. Agr. Ann. Appl. Biol. Bull. Circ. Coll. Expt. J. Physiol. Plant Disease Reptr. Proc. Research Sta. One-word journal names are always in full, e.g., *Phytopathology*.

Order of paper.—Follow the order above. Text will be followed by: tables; Fig. captions; any footnotes.[1]

Phytopathological notes.—All comments herein apply equally to Phytopathological Notes, except that Notes have no summary. Phytopathological Notes are brief accounts (1–4 typewritten pages) needing no detailed treatment. They may deal with research, matters of historical or current interest, comment on published papers, or discussion of scientific concepts.

Submissions.—Send mss. and correspondence to Editor-in-Chief. At least 1 author must be a member of the American Phytopathological Society, except that each subscribing institution outside U. S. and Canada is allowed 1 published paper by nonmembers in any 1 volume of *Phytopathology*. English is required.

Subject matter must be: 1) unpublished original research; 2) review papers developing a new concept, hypothesis, theory, or other integration of the science; or 3) Phytopathological Note (see above). Submission implies nonsubmission elsewhere and (if accepted) no publication elsewhere in same form without consent.

[1]Avoid footnotes. They are distracting and costly. Give the information in the text (in parentheses).

Table 1.—Abbreviations used. Typists please follow if authors do not. *Never* use vertical lines in tables.[a]

Heading	Heading	Heading	Heading
* significant at 5% level	cu cubic	LD_{50} lethal dose, 50%	oz.[b]
** significant at 1% level	ED_{50} effective dose, 50%	log	p.[b] page
μ micron	et al	LSD least significant difference	p.m.[b]
% per cent	°F	m meter	ppm
A.[b] acre	Fig.[b]	m milli-	psi lb/sq. in.
A Angstrom	ft	M[c] molarity	pt
a.m.[b]	ft-c	me milli-equivalent	qt
amp	g gram	N[c] normality	rep roentgen-equivalent-physical
atm	G gravity	no.[b]	RH relative humidity
bu	gal.[b]	ns not significant	rpm
°C	in.[b]	W watt	sq
cc	kg		
cm	L liter		
	lb		

[a]Note 2 general rules: 1) don't add *s* for plurals; 2) superscripts for table footnotes are *letters*.

[b]Note that only 9 items have periods.

[c]Note that only 2 items are underlined: in addition, these 2 follow numbers *without* spacing, e.g., 6N HCl.

Chemical nomenclature.—Since formulas change, trade or code names must be followed by chemical names of active ingredients, with any percentages less than 100%. For chemical names of fungicides, see *Phytopathology* 45:295-302.

Your edited ms.—Our main editorial aims are to: 1) promote precision and reading ease; 2) cut waste words and overexplanation; 3) follow established style.

Sense in style.—Many are unaware that grammarians agree on the following: 1) *None* is usually plural, meaning *not any*; use it as singular only to emphasize *not one*. 2) Adverbs *should* split compound verbs unless stress requires other placement. 3) Collective nouns may be plural, and often are. 4) *A number of* means *several*, and is plural. 5) Waste words waste money and time; throw them out. 6) Parallel ideas or like findings should be expressed in like ways; varying the structure or words for style is confusing.

Special to secretaries.—*Manuscript form.*—Type with fresh ribbon on 1 side of white bond ($8\frac{1}{2}\times11$-in. preferred). Send original and 1 *good* carbon; keep a second against possible loss. Identify each page with authors' names at top.

Indenting.—*All* first lines are indented. That includes paragraphs, section headings, figure captions, table headings, Literature Cited, footnotes—*everything!*

Spacing.—Double-space everything—i.e., *everything!*

Headings.—Only SUMMARY and LITERATURE CITED are centered and capitalized. All others are in lower-case and indented as paragraph beginnings:

Main headings.—*Second rank of heading.*—1) *Third rank.*—a) *Fourth rank.*—String them together if more than 1 come together. Underline as indicated.

Fig. 1.—Your figures will appear either 1 or 2 columns wide. Indicate which preferred. If Fig. caption indicates magnification, indicate it for the size as it will be printed. Protect photographs (glossy prints preferred) against wrinkling; mount them on a heavy board with smooth white surface ($8\frac{1}{2}\times11$ in. preferred). Identify each by author, title, and Fig. no. Combine those you can—and *don't* waste space between combined figures. Put all Fig. captions on 1 sheet.

Author pays $\frac{1}{2}$ of illustration costs exceeding $16 (about 1 full page or two $\frac{1}{4}$ pages). Line drawings serving as tables are not charged for unless, with tables, they exceed 30% of article length.

INFLUENCE OF SOIL TEMPERATURE ON SEEDLING BLIGHT OF SMOOTH BROMEGRASS

Ivan J. Thomason and J. G. Dickson

Respectively, former graduate Assistant in Plant Pathology, University of Wisconsin, now Assistant Nematologist, University of California, Riverside; and Professor of Plant Pathology at the University of Wisconsin.

Accepted for publication June 11, 1959.

A portion of a thesis submitted in partial fulfillment of the requirements for the Ph.D degree, University of Wisconsin.

SUMMARY

Seedling blight of smooth bromegrass has been a major factor in establishing field stands. The influence of environment on disease development was investigated in relation to: 1) natural infestation with blight-inciting fungi of soils varying widely in type, crop sequence, and location, 2) isolation and identification of the fungi present in the diseased seedling tissues, and 3) ability of these fungi to incite disease over the range of environment studied. *Pythium*, *Fusarium*, *Helminthosporium*, and *Rhizoctonia* spp., in descending order of prevalence, were studied. Disease present in brome seedlings representative of southern and northern ecotypes, grown at 4 temperatures and at 2 or more soil moisture levels in infested soils and in pure culture inoculations, indicated that low soil temperature and medium soil moisture were essential to establishing healthy plants. Soil temperature influenced postemergence blighting more than preemergence blighting of bromegrass seedlings. Postemergence blighting was severe only at 28° and 24°C. Soil origin and fungus flora were important in determining the severity of pre-emergence injury. *Pythium* spp. and *Helminthosporium sativum* were associated with greater disease expression than *Rhizoctonia solani* and *Fusarium* spp.

Introduction.—Smooth bromegrass, *Bromus inermis* Leyss, is an increasingly important forage grass in the North Central and Great Plains states. The establishment of field plantings is often difficult.

Stand failures occur in both late summer and spring plantings. Seedlings emerge, grow for several days or weeks, and then blight. In many cases the death of the seedlings is associated with a brown to black necrosis of the hypocotyl and roots. Older seedlings usually show more tolerance to damage by fungi.

Numerous species of fungi are isolated from the root and crown tissues of cereals and grasses. A relatively small number of these are shown to be primary pathogens. The remainder are either secondary invaders or essentially saprophytic in behavior.

Seed-rot and seedling blight of cool-season grasses has been attributed to *Pythium graminicola* Subr. and other fungi (13, 14). A total of 949 isolations were made from bromegrass by Sprague (13) in 1940–43: *Fusarium roseum* Lk. was the most prevalent, followed in order by *Pythium* spp., *Helminthosporium sativum* (Pam.) King & Bakke, *Fusarium oxysporum* Schlecht., and *Rhizoctonia solani* Kühn. *P. graminicola* was the most prevalent *Pythium* species. In a survey of *Fusaria* associated with root rots of Graminaceae in the Northern Great Plains, Gordon and Sprague (5) obtained 90 isolates from bromegrass—60 in the *F. roseum* group and 30 identified as *F. oxysporum*.

Accumulated evidence indicates that seedling blight of grasses is most severe in warm soils. It is well known that soil temperatures can be quite high in spring and fall as well as in summer (9). The influence of soil temperature on the pathogenicity of root disease fungi has been demonstrated by many investigators. The literature on this subject was reviewed by Garrett (4).

The development of seedling blight of created wheatgrass, *Agropyron cristatus* (L.) Beauv., planted in field soils in the greenhouse, was studied by Buchholtz (2) in South Dakota. He found that low soil temperature delayed seedling growth and onset of blight. At higher temperatures, seedling growth was more rapid but blight appeared sooner and final stand losses were greater.

Low soil temperature (21°C) was reported by Hawk and Welch (6) to favor stand establishment of bromegrass in soil infested with *Pythium graminicola*. They also concluded that the southern ecotype of bromegrass, as represented by the varieties Achenbach, Fischer, and Lincoln, was more tolerant to *Pythium* root rot than was the northern ecotype, as represented by Canadian Common, Manchar, and Parkland.

Andrews (1) reported that the pathogenicity of *P. graminicola* on bromegrass and crested wheat grass was similar at soil temperatures in the range 12–32°C.

The experiments reported here were made to study the influence of soil temperature on the development of seedling blight of bromegrass. Tests were conducted first in naturally infested field soils. Isolations were made from diseased seedlings to determine the kind and relative prevalence of fungi attacking bromegrass. The ability of several fungi to incite seedling blight at several soil temperatures was then tested in the greenhouse.

Seedling blight from naturally infested soils.—*Materials and methods.*—Three experiments were designed and conducted to determine the influence of soil-type, temperature, and moisture on the development of seed-rotting and seedling blight of bromegrass. All experiments were conducted in the winter at air and soil temperatures of 28, 24, 20, and 16°C.

1) Canadian Common bromegrass (50 seeds per pot) was planted in nonsterile peat-muck soil and in white silica sand in 6-in. glazed earthenware crocks. Soil moisture was maintained at optimum for seedling growth. In the sand, moisture and nutrients were supplied by watering with a 1*N* Hoagland's solution at pH 5.7.

2) Canadian Common bromegrass seedlings were grown in 5 naturally infested soils at optimum mois-

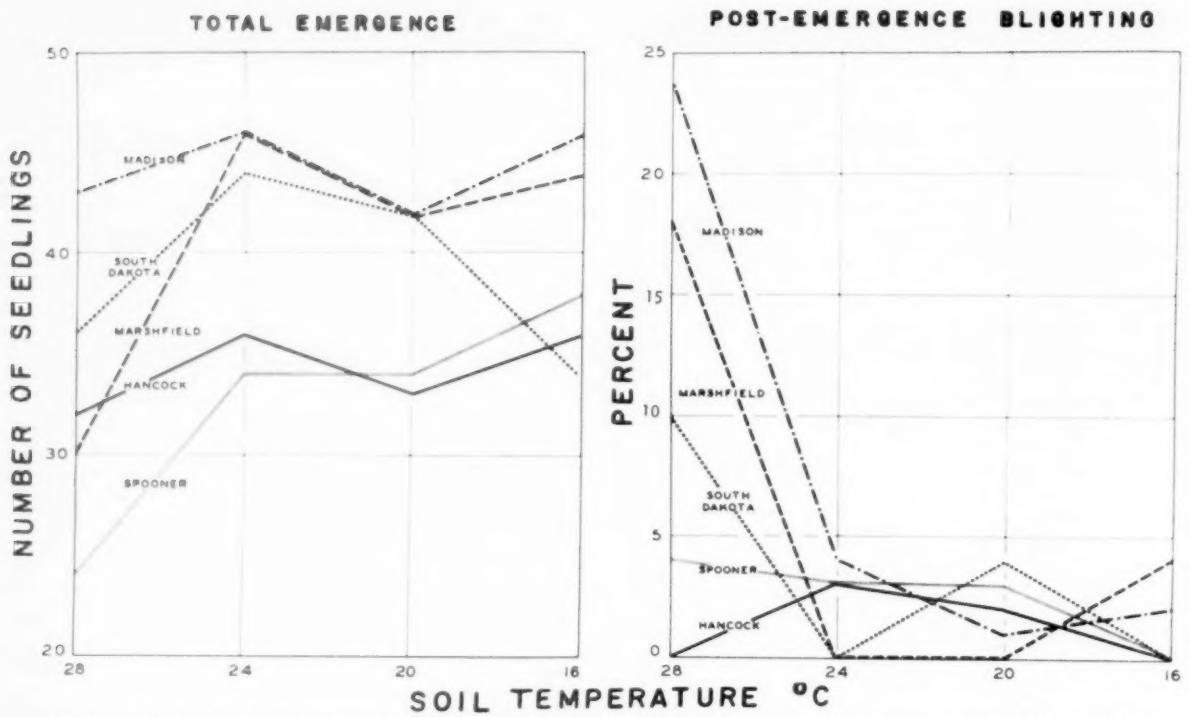


Fig. 1. The influence of temperature on total emergence and postemergence blighting of Canadian Common bromegrass seedlings in 5 naturally infested soils. Average of 3 replications, 50 seeds per pot.

ture. The soil types and the areas from which they were obtained are as follows: Plainfield sand, Hancock; Peat-Muck, Madison; Colby silt loam, Marshfield; Omega sand, Spooner, Wisconsin; and Boyd-Holt sandy loam, Brookings, South Dakota. The range of pH in all soils was 5.3–5.7. The soil from South Dakota was representative of an area in which the establishment of grass stands was especially difficult.

3) The soil from Brookings was used in further tests designed to evaluate the influence of soil moisture level as well as soil temperature on seed rot and seedling blight of Canadian Common bromegrass. Two soil moisture levels were maintained: near saturation (13.5% by dry weight of the soil) and optimum (10% by dry weight).

The number of seedlings damping-off during the course of the experiments was recorded, and the remaining seedlings were removed from the soil and rated for disease development 3–5 weeks after planting. The analysis of variance was used as an aid in evaluating the data.

Results.—Daily records were kept on emergence of the seedlings in preliminary tests of the influence of soil temperature on the growth of Canadian Common bromegrass. Five weeks after planting final readings were taken of emergence, height of tops, length of roots, and green weight of plants. Total emergence was similar at the 4 soil temperatures, but rate of emergence was influenced by soil temperature.

The rate of plant development was influenced by soil and air temperature. Height of the plants was not significantly affected in the ranges 28–24° and 20–16°C, but was significantly reduced in the range 24–20°C. Roots were not significantly shortened between 28° and 20°C, but they were shorter at 16°C. Although a reduction in plant size was evident at 16°C, the seedlings showed a better ratio of roots to tops and less root necrosis.

In the second test with the 5 naturally infested soils, final counts on total emergence and postemergence blighting were made 21 days after planting. Soil type and temperature affected both total emergence and the amount of postemergence blighting of seedlings (Fig. 1). Total seedling emergence was lowest in the sandy soils from Hancock and Spooner. Poor emergence in these soils was associated with the isolation of large numbers of *Pythium* spp. In 4 of the 5 soils, emergence was lowest at 28°C. Except in the South Dakota soil, in which emergence fell off sharply at both 28° and 16°C, emergence was generally markedly reduced at 28° and 20°C.

Postemergence blighting, which was relatively low in this experiment, was most severe at 28°C in 3 of the 5 soils. Postemergence blighting was generally severe in soils having highest emergence. Postemergence blighting was not severe in the two sandy soils

Fig. 2. Fungi isolated from the root and crown tissues of Canadian Common bromegrass seedlings grown in 5 naturally infested soils. Each fungus at each of 4 soil temperatures is presented as a percentage of the total fungi isolated from that particular soil.

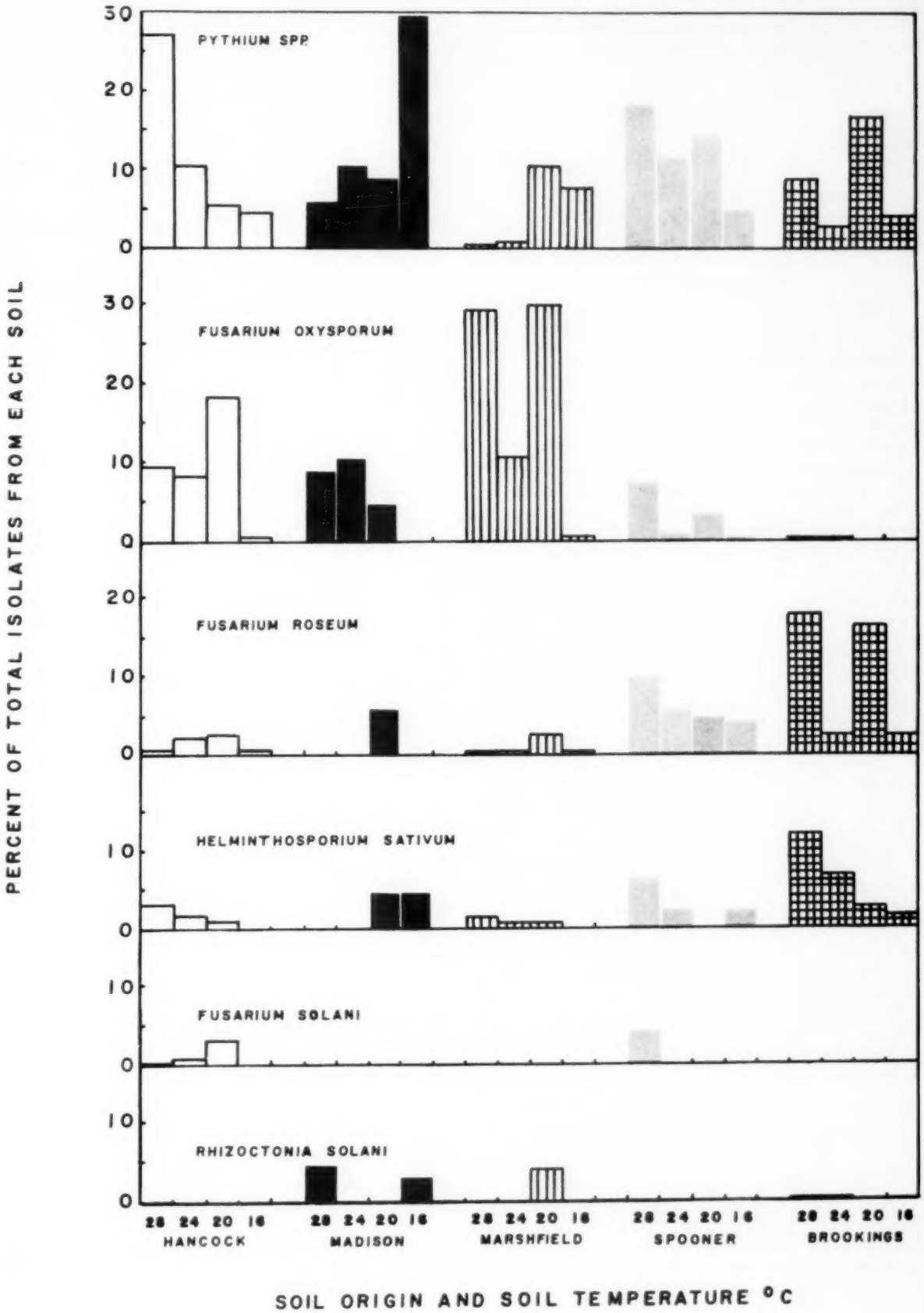

PERCENT OF TOTAL ISOLATES FROM EACH SOIL
PYTHIUM SPP.
FUSARIUM OXYSPORUM
FUSARIUM ROSEUM
HELMINTHOSPORIUM SATIVUM
FUSARIUM SOLANI
RHIZOCTONIA SOLANI
0
10
20
30
28 24 20 16
HANCOCK
MADISON
MARSHFIELD
SPOONER
BROOKINGS
SOIL ORIGIN AND SOIL TEMPERATURE °C

TABLE 1.—*Prevalence of various fungi obtained from root and crown tissues of bromegrass seedlings growing in naturally infested soils*

Fungus	No. times isolated	% of total isolated
Pythium spp.	913	32.7
Fusarium roseum	608	21.8
Fusarium oxysporum	489	17.5
Helminthosporum sativum	327	11.7
Rhizoctonia solani	61	2.2
Fusarium solani	38	1.4
Curvularia geniculata	16	0.6
Determined miscellany	157	5.6
Undetermined miscellany	179	6.4
Total	2788	

where total emergence was low.

Injury to the coleoptilar tissue and roots of the seedlings was most severe in all soils at 28°C, and decreased at the lower temperatures. At 16°C very little damage to the coleoptile or roots was evident.

Neither soil temperature nor moisture level significantly influenced total emergence of the bromegrass seedlings in the test in soil from Brookings, South Dakota. Emergence approached 89% of that expected at all temperatures. However, postemergence blighting was severe and soil temperature had a marked influence on the development of the blighting. At 28°C, 32% of the seedlings at the optimum moisture level damped-off and 24% of those in the saturated soil. At 24°C, 14% of the seedlings at both moisture levels were killed. No postemergence blighting occurred at 20° and 16°C.

The results of the 3 tests indicated that bromegrass germinated and developed best in soils at 24°, 20°, and 16°C. Although emergence and development of the seedlings was slower at 20° and 16°C, less root injury was observed. Some pre-emergence blighting occurred in some soils at these lower temperatures, but postemergence blighting was not severe.

Fungi isolated from diseased seedlings of bromegrass grown in naturally infested soils.—*Materials and methods.*—Bromegrass seedlings were grown in 5 naturally infested soils at soil temperatures of 28, 24, 20, and 16°C to determine the species and relative prevalence of fungi inciting disease under these varying conditions. Sections of roots and crowns from seedlings showing the several symptoms in each soil and temperature were placed in cheesecloth bags and washed 5–8 hours in running tap water. The sections were removed from the bags, rinsed in sterile water, and placed on water agar for 2–3 days, and mycelial segments from each fungus colony were transferred to potato-cerelose agar. In the test with the 5 naturally infested soils, random samples of diseased bromegrass seedlings were taken and isolations were made at several intervals during the growth of the plants and at the end of the experiment. Fungi isolated from the seedlings in the different soils, and from the 4 temperatures, were recorded separately.

Experimental results.—The fungi were identified from the cultures growing on nutrient agar. *Pythium* isolates were classified under the major groups occurring commonly on this type of diseased tissue, principally *Pythium debaryanum* Hesse and *P. graminicola* (*P. arrhenomanes* Drechsl.). The Snyder and Hansen (10, 11, 12) system of classification was used for the *Fusarium* species, although several distinct morphological types were differentiated in the *F. roseum* group. Ninety per cent of the isolates in this group were of the type previously called *Fusarium equiseti* (Cda.) Sacc.

The isolations yielded many different fungus species, most of which were identified as to genera and species. A group of "determined miscellany" included fungi belonging to the following genera: *Aspergillus*, *Chaetomium*, *Mucor*, *Phoma*, *Sclerotium*, *Trichoderma*, *Penicillium*, and a *Basidiomycete*. Another group, "undetermined miscellany," included nonsporulating types, some of which produced dark mycelial strands in the agar.

The fungi isolated from bromegrass seedlings grown in the 5 naturally infested soils are summarized by graph in Fig. 2. As might be expected, origin of the soil appeared to be the most important factor in determining fungi isolated. Cropping history, soil textures, and pH are factors that might be expected to have an influence on the fungus population of the various soils. *Pythium* spp., *Fusarium roseum*, and *Helminthosporium sativum* were isolated from seedlings grown in all soils at most temperatures. *Fusarium oxysporum* was isolated only rarely from any of the seedlings grown at a soil temperature of 16°C. In addition, fewer isolates of *H. sativum* were normally recovered at the lower temperatures. The occurrence of other fungi did not appear to be strongly influenced by soil temperature.

All the fungi isolated from bromegrass seedlings in the 3 tests described in the previous section are listed in Table 1. *Pythium* spp. were by far the most numerous. When isolated from seedlings that had damped-off after emergence, the isolates most commonly had the cultural characteristics of *P. graminicola*. Most of the *Pythium* isolates from seedlings grown in the Madison peat soil at 16°C were *P. debaryanum*. *Fusarium oxysporum* was isolated only from several seedlings grown in soil from Brookings, South Dakota, in the test reported in Fig. 4, and was recovered in subsequent tests with the Brookings soil.

Influence of soil temperature on pathogenicity of fungi from bromegrass.—*Materials and methods.*—The several fungi commonly isolated from the bromegrass seedlings were evaluated for pathogenicity on Canadian Common and Lincoln varieties of bromegrass grown at temperatures from 12 to 28°C in sterilized sandy-loam soil or sand and 1/4 Hoagland's solution. The inoculum used was prepared by removing the mycelial mat from fresh cultures on nutrient media, fragmenting 15 seconds in a Waring blender, and incorporating the suspension directly into the soil or into sterile sand that was then mixed

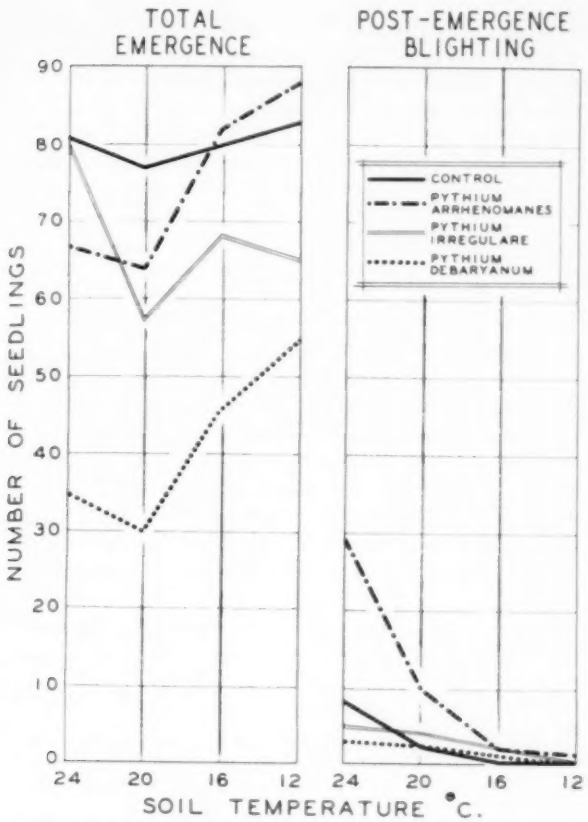


Fig. 3. Relative pathogenicity of 3 *Pythium* spp. on Canadian Common bromegrass at 4 soil temperatures. Average of 3 replications, 100 seeds per pot.

with soil or sand. The randomized complete block design used for the several experiments permitted comparative evaluation of the pathogenicity of the fungi on the 2 hosts at 4 different temperatures, and the general effect of temperature on host and disease development. Cultural procedures, data taken, and isolation of fungi were similar to those described previously.

Experimental results.—Pythium graminicola, P. irregulare, and P. debaryanum[1] differed markedly in pathogenicity to bromegrass seedlings as measured by total emergence and postemergence blighting in a sandy-loam soil at temperatures of 24, 20, 16, and 12°C (Fig. 3). Total emergence was reduced in soils infested with *P. debaryanum* at all soil temperatures, but was lowest at 20 and 24°C. Pre-emergence blighting occurred only at 20° and 24°C in *P. graminicola*-infested soil, and postemergence blighting was also severe. In soil infested with *P. irregulare* at 20, 16, and 12°C, pre-emergence blighting occurred, as indicated by total emergence figures, but postemergence blighting was not severe. The results indicated that injury was more serious at higher soil temperature. Consequently the upper limit of the temperature series was shifted to 28°C in subsequent tests.

[1] Cultures isolated originally from corn seedlings by P. E. Hoppe.

Severe pre-emergence blighting of both Canadian Common and Lincoln bromegrass occurred at 28, 24, 20, and 16°C when these hosts were grown in sand infested with a bromegrass isolate of *P. graminicola*. Both varieties showed blighting, but emergence was reduced more in Lincoln than in Canadian Common. The average reduction in emergence for all 4 temperatures was 78% in Lincoln and 38% in Canadian Common (Fig. 4). In another test with Canadian Common under similar conditions pre-emergence blighting was not as severe (an average of 14% for all 4 temperatures). At 28°C a large percentage of the seedlings that did emerge damped-off (Fig. 4). At the lower temperatures, damping-off was less severe and did not occur at 16°C. The pathogenicity of 6 isolates of *H. sativum*, 3 from barley and 3 from bromegrass, was compared on the 2 varieties of bromegrass grown in sand at 22 and 24°C air temperature. Final readings were taken on total emergence, height of tops, and length of roots, 23 days after planting. Plants were placed in 5 classes from 0 to 10, according to severity of injury, and a disease index calculated. Data are presented in Table 2. The origin of the *H. sativum* culture, i.e., whether from barley or bromegrass, did not influence pathogenicity significantly. One isolate from barley (Ba-1-53) and one from bromegrass (Br-6-53) were more pathogenic than the others. From reductions in emergence and the disease index, the variety Lincoln appeared more susceptible than Canadian Common.

The pathogenicity to Lincoln bromegrass of *H. sativum* isolate Br-6-53 was tested further in sand at temperatures of 28, 24, 20, and 16°C. Emergence of Lincoln bromegrass seedlings was reduced an average of 30% for all temperatures in sand infested with *H. sativum* (Table 3). Pre-emergence blighting was not influenced by soil temperature. Postemergence blighting (20%) was serious only at 28°C. However, some injury to the emerged seedlings was evident at all temperatures. Stunting of seedlings and necrosis of the roots and coleoptile were most severe at 28 and 16°C. Although both pre- and postemergence blight-

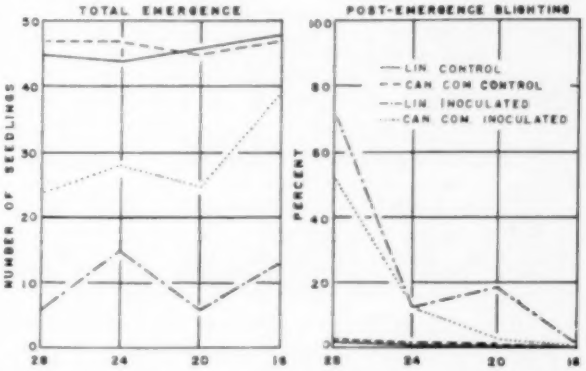


Fig. 4. Total emergence and postemergence blighting of Canadian Common and Lincoln bromegrass seedlings at 4 temperatures (°C) in sand infested with *Pythium graminicola*. Average of 3 replications, 50 seeds per pot.

TABLE 2.—*Relative pathogenicity of six isolates of Helminthosporium sativum to Lincoln (Lin.) and Canadian Common (C.C.) bromegrass seedlings grown in 22° soil at 24°C air temperature*[a]

		Barley isolates				Bromegrass isolates			
		Check	Ba–1–53	Ba–2–53	Ba–13–53	Br–6–53	Br–14–53	Br–15–53	Av.
Number of seedlings	Lin.	46	26	40	33	22	36	38	34
	C.C.	46	38	44	38	33	46	45	42
Height of tops (cm)	Lin.	15.7	9.7	14.3	11.5	7.8	14.7	11.5	12.2
	C.C.	20.0	13.5	19.3	15.0	12.0	15.5	19.0	16.3
Length of roots (cm)	Lin.	12.3	5.3	9.5	5.8	3.8	6.0	5.2	6.8
	C.C.	11.7	5.3	11.3	6.5	5.3	6.0	7.7	7.1
Disease index[b]	Lin.	7	67	54	62	62	34	52	48
	C.C.	8	54	33	48	60	30	40	39

[a] Data average of 3 replications, 50 seeds per pot.
[b] Disease index: 0 = all healthy, 100 = all seedlings dead.

ing occurred in the presence of *H. sativum*, it was not as severe as that associated with *P. graminicola*.

The 2 isolates of *R. solani* obtained from bromegrass that were compared did not cause pre-emergence blighting of Canadian Common and Lincoln seedlings at any of the 4 soil temperatures. Some postemergence lesioning and blighting occurred, but it was not serious. Postemergence blighting was most severe at 28°C with one isolate of the fungus, but not strongly influenced by temperature with the other. Canadian Common was injured somewhat more than was the variety Lincoln. Injury to the bromegrass seedlings was confined, for the most part, to necrosis of the coleoptilar tissue at the surface of the sand. Very little root injury was observed, although the roots were found to be closely associated with the fungus mycelium.

The 5 *Fusarium* isolates compared for their pathogenicity to Lincoln bromegrass at 4 soil temperatures were obtained from the roots of bromegrass seedlings grown in the field at Hancock, Wisconsin. Two isolates of *F. roseum* differing in gross morphological characteristics were used, one of *Fusarium tricinctum* (Cda.) Sacc. ex Snyd. & Hans., and one each of *F. oxysporum* and *F. solani*. The data are presented in Table 4. None of the 5 *Fusarium* isolates caused severe pre-emergence blighting. *F. tricinctum* and one isolate of *F. roseum* reduced stand slightly, but were relatively innocuous in comparison with *P. graminicola* and *H. sativum*.

TABLE 3.—*Influence of soil temperature on the pathogenicity of Helminthosporium sativum (isolate Br–6–53) on Lincoln bromegrass*[a]

Soil temperature (°C)	Total emergence (no. seedlings)		Post-emergence blighting (no. seedlings)		Post-emergence blighting (%)	
	Control	Br–6–53	Control	Br–6–53	Control	Br–6–53
28	46	32	1	6	2.2	18.8
24	44	31	1	1	2.3	3.0
20	44	32	0	1	0.0	3.0
16	46	31	0	0	0.0	0.0
Average	45	31.5	0.5	2.0	1.1	6.2

[a] Data average of 3 replications, 50 seeds per pot.

Discussion.—Important considerations in the development of bromegrass seedlings in infested soil were condition of the seed and its capacity to germinate and grow vigorously. Preliminary trials indicated that response to seed treatment was greatest in seed lots of low vigor. Seed treatment improved emergence but did not protect the seedlings from postemergence blighting.

Dickson (3) and Leach (7) found that pre-emergence blighting and seedling injury were more serious when crop plants were grown at soil temperatures that favored growth of the pathogen more than growth of the host. This may explain, in part, why post-emergence damping-off of bromegrass was more serious at high temperatures since the optimum temperature for growth of *P. graminicola* and *H. sativum* was about 28°C, whereas bromegrass seedlings showed a physiologically better balanced growth at temperatures of 20 and 16°C.

Canadian Common and Lincoln bromegrass, respectively representing the northern and southern ecotypes, apparently responded differently to pathogens and temperature. With the particular seed lots used, Canadian Common showed better stands in the presence of *P. graminicola* and *H. sativum*, especially at the low temperatures, than Lincoln. This is in contrast to the results obtained under field conditions by Hawk and Welch (6) and Nielson, Dickson, and Smith (8), who concluded that varieties representing the southern ecotype were more resistant to seedling blight

TABLE 4.—*Average emergence of Lincoln bromegrass seedlings in sand infested with 5 Fusarium spp. at 4 soil temperatures*[a]

Fusarium spp.	Total emergence (no. seedlings)
Control	42.8
Fusarium acuminatum	39.1
Fusarium equiseti	44.4
Fusarium poae	40.2
Fusarium oxysporum	42.1
Fusarium solani	45.1
L.S.D.	.05, 2.2
L.S.D.	.01, 2.9

[a] Data average of 3 replications at each of 4 soil temperatures, 50 seeds per pot. Soil temperatures, 28, 24, 20, and 16°C.

than were the northern. The plants of the southern ecotypes, through the process of natural selection, may show more resistance to seedling blight; however, in the limited comparisons reported here, the northern ecotypes appear to show better plant development and less disease at the low temperatures than the southern.

Pythium spp. were the more common fungi isolated from diseased seedlings. Although *Fusarium roseum* and *Fusarium oxysporum* were isolated from diseased seedlings they did not cause serious injury under the conditions of these tests. *Rhizoctonia solani* was not commonly isolated and caused only a small amount of postemergence blighting. *Helminthosporium sativum* made up 11% of the isolates from bromegrass seedlings. Isolates of *H. sativum* from bromegrass and barley were equally pathogenic to bromegrass seedlings.

The results of these tests indicated that stands of bromegrass were better in soils at temperatures at or below 20°C.—Department of Plant Pathology, University of Wisconsin, Madison.

LITERATURE CITED

1. ANDREWS, E. A. 1953. Seedling blight and root rot of grasses in Minnesota. Phytopathology **33**: 234-239.
2. BUCHHOLTZ, W. F. 1949. A comparison of gross pathogenic effects of Pythium graminicola, Pythium debaryanum, and Helminthosporium sativum on seedlings of crested wheatgrass. Phytopathology **39**: 102-116.
3. DICKSON, J. G. 1923. Influence of soil temperature and moisture on the development of the seedling blight of wheat and corn caused by Gibberella saubinetti. J. Agr. Research **23**: 837-870.
4. GARRETT, S. D. 1944. Root Disease Fungi. Chronica Botanica Company. Waltham, Mass. 177 p.
5. GORDON, W. L., AND R. SPRAGUE. 1941. Species of Fusarium associated with rootrots of the Gramineae in the Northern Great Plains. Plant Disease Reptr. **25**: 168-180.
6. HAWK, V. B., AND A. W. WELCH. 1948. Tolerance of varieties of smooth bromegrass, Bromus inermis Leyss., to injury by rootrot, Pythium graminicola Subr. J. Am. Soc. Agron. **40**: 809-817.
7. LEACH, L. D. 1947. Growth rates of host and pathogen as factors determining the severity of preemergence damping-off. J. Agr. Research **75**: 161-179.
8. NIELSEN, E. L., J. G. DICKSON, AND D. C. SMITH. 1959. Strain and seed treatment as factors in germination and seedling growth of smooth bromegrass. Phytopathology **49**: 8-12.
9. SHAW, B. T., ed. 1952. Soil physical conditions and plant growth. (Agronomy 2) Academic Press, N. Y. 491 p.
10. SNYDER, W. C., AND H. N. HANSEN. 1940. The species concept in Fusarium. Am. J. Botany **27**: 64-67.
11. SNYDER, W. C., AND H. N. HANSEN. 1941. The species concept in Fusarium with reference to section Martiella. Am. J. Botany **28**: 738-742.
12. SNYDER, W. C., AND H. N. HANSEN. 1945. The species concept in Fusarium with reference to discolor and other sections. Am. J. Botany **32**: 657-666.
13. SPRAGUE, R. 1944. Rootrots of cereals and grasses in North Dakota. N. Dakota Agr. Expt. Sta. Bull. 332 (Tech.). 35 p.
14. SPRAGUE, R. 1946. Rootrots and leafspots of grains and grasses in the Northern Great Plains and Western States. Plant Disease Reptr. Suppl. 163.

A STRAIN OF CUCUMBER VIRUS 1 INFECTIOUS TO BLIGHT-RESISTANT SPINACH

S. P. Doolittle and R. E. Webb

Pathologist, Crops Research Division, ARS, U. S. D. A., Beltsville, Maryland.

Accepted for publication June 12, 1959.

SUMMARY

A virus isolated from *Commelina nudiflora* that mottles, dwarfs, and kills young plants of spinach varieties resistant to a number of strains of cucumber mosaic virus 1, has been identified as another strain of this virus. Its symptoms on certain hosts distinguish it from the typical strain, as well as from two other strains of cucumber virus 1 also highly infectious on spinach.

In screening selected lines of spinach for resistance to blight caused by cucumber virus 1 (CMV) Webb et al. (3) used the typical strain and 6 atypical strains from a collection maintained by the senior author on caged plants of Turkish tobacco in a greenhouse at the Plant Industry Station, Beltsville, Md. Five of the atypical strains and the typical strain produced no symptoms on a highly resistant selection (P.I. 179590) from a recent spinach introduction from Belgium. The sixth strain was highly infectious to spinach and severely damaged or killed all the plants of the resistant selection and those of resistant and susceptible commercial spinach varieties and hybrids tested. In more recent tests all plants of 120 additional plant introductions were also severely injured.

This last virus was collected in 1950 by Freeman A. Weiss on plants of day flower, *Commelina nudiflora* L., at the Plant Industry Station. It was given to the senior author, and, after test inoculations on cucumber, tobacco, tomato, and a few other species, was classed as an isolate of CMV and maintained on Turkish tobacco in the greenhouse.

The recent discovery of the lethal effect of the commelina strain on plants of spinach varieties and lines resistant to a number of other strains of CMV suggested the advisability of using this strain in screening spinach breeding lines for blight resistance. There are grounds for a belief that lines resistant to this strain are likely to be resistant to many other known strains of CMV (3). The physical properties of the commelina strain and its distinguishing symptoms on certain hosts are described in this paper.

Materials and methods.—In these studies, all inoculations were made on young plants in the greenhouse at temperatures ranging from 22 to 28°C. The typical strain and the commelina strain were maintained on young, rapidly growing Turkish tobacco plants. Inoculum was prepared by macerating infected

leaves in a small amount of water. Inoculations were made by dusting leaves with carborundum and rubbing them with a cheesecloth pad saturated with the inoculum. Plants were rinsed with water after inoculation.

Symptoms and host range.—The most important differential characteristic of the commelina strain was its ability to cause severe injury to spinach varieties resistant to all the other strains of CMV tested. However, the symptoms produced by the commelina strain differed markedly from those produced by the typical strain on a number of other hosts also. On hosts on which the symptom patterns of both strains were much the same the mottling caused by the commelina strain tended to be more pronounced.

The resistant spinach varieties Old Dominion, Virginia Savoy, and a highly resistant selection from P.I. 179590 were not affected by the typical strain. When inoculated with the commelina strain, young plants having 4–6 leaves showed rapid yellowing, stunting, and necrosis of the younger leaves. These plants collapsed and died within 8–12 days. Plants of the blight-susceptible variety Bloomsdale were yellowed, stunted, and killed by the commelina strain, but no more rapidly than were the resistant varieties just mentioned. The typical strain also yellowed and stunted Bloomsdale plants and eventually killed a few. Disease development in all varieties was more rapid above 24°C.

The two strains also showed pronounced differences in their effects on certain hosts other than spinach. These hosts and their symptoms are as follows.

Cucurbitaceae.—1) *Cucumber, Cucumis sativus L. var. Marketer. Commelina strain:* Stems of seedlings often shriveled and killed. Faint yellow primary lesions on cotyledons. Bright-yellow vein clearing and yellow dots on leaves first showing systemic symptoms. Later ones smooth and mottled with small yellow patches. Sometimes V-shaped necrotic patches on old leaves. *Typical strain:* Seedlings rarely killed. No primary lesions on cotyledons. Mild vein clearing on leaves first showing systemic symptoms. Later ones mottled with large yellow patches. Green areas raised and blister-like. No leaf necrosis.

2) *Muskmelon, Cucumis melo L. var. Hearts of Gold. Commelina strain:* Pale-tan primary lesions on cotyledons. Strong yellow vein clearing and mottling of leaves first showing systemic symptoms. Pronounced yellow mottling of later growth. *Typical strain:* No primary lesions on cotyledons. Faint vein clearing of leaves first showing systemic symptoms and a mild yellowish-green mottling of later ones.

3) *Squash, Cucurbita pepo L. var. Early Prolific Straightneck. Commelina strain:* Large (3–5 mm) brown, circular primary lesions on cotyledons. Leaf symptoms similar to those on muskmelons. Plants stunted more than by typical strain. *Typical strain:* Symptoms similar to those on muskmelon but more pronounced yellow-green mottling of later leaves.

Solanaceae.—1) *Tobacco, Nicotiana tabacum L. var. Samsun. Commelina strain:* Grayish-white, etched, circular primary lesions 3–5 mm in diameter. Brilliant yellow vein clearing on leaves first showing systemic symptoms followed by mottling with sharply defined yellow-green spots. Mild yellow-green mottling of later growth. Little distortion of leaves. *Typical strain:* No leaf lesions. Mild pale-green vein clearing on leaves first showing systemic symptoms. Mild light- and dark-green mottling of later growth. Dark areas often blister-like and some leaves misshapen.

2) *Nicotiana glutinosa L. Commelina strain:* Faint yellowish rings and strong vein clearing on inoculated leaves. Light- and dark-green stippled mottling and occasional filiformity on later growth. *Typical strain:* Faint vein clearing and yellowish-green mottling on inoculated leaves. Yellowish-green mottling of later growth. No leaf deformity.

Leguminoseae.—1) *Bean, Phaseolus vulgaris L. vars. Refugee and Pinto. Commelina strain:* Pronounced pale-green systemic mottling of leaves of Refugee. Yellowing and veinal necrosis of inoculated primary leaves followed by systemic green mottling of trifoliolate leaves of Pinto. *Typical strain:* No symptoms on Refugee. Brown stipple necrosis of inoculated primary leaves of Pinto, but no systemic symptoms.

2) *Broad bean, Vicia faba L. var. minor Peterm. var. Bell Bean. Commelina strain:* Small, chocolate-brown primary lesions. Mild green systemic mottling. *Typical strain:* Sparse, brown, necrotic primary lesions. No systemic symptoms.

3) *Cowpea, Vigna sinensis (Torner) Savi var. Black. Commelina strain:* Small, brown primary lesions and veinal necrosis on primary leaves. Pronounced systemic yellow mottling of trifoliolate leaves. *Typical strain:* Small, brown primary lesions. No systemic symptoms.

4) *Pea, Pisum sativum L. var. Thomas Laxton. Commelina strain:* Mild, pale-green systemic mottling. Yellowing and withering of older leaves. *Typical strain:* No symptoms.

Both strains produced similar symptoms on all the other species tested except Tepary bean, *Phaseolus acutifolius* var. *latifolius* Freeman, which was not infected by either. On many of these hosts the commelina strain caused more pronounced mottling of the leaves and slightly more stunting of the plants, but the systemic symptoms were not markedly different from those caused by the typical strain. The same was true of primary lesions if these occurred. The host species in this group were as follows: *Antirrhinum majus* L. (snapdragon), *Apium graveolens* L. (celery), *Beta vulgaris* L. (garden beet), *Capsicum frutescens* L. (sweet pepper), *Datura stramonium* L. (jimson weed), *Gomphrena globosa* L. (globe amaranth), *Lycopersicon esculentum* Mill. (tomato), *Nicotiana rustica* L., *N. sylvestris* Speg., *Petunia hybrida* Vilm. (petunia), *Phaseolus lunatus* L. (lima bean), *Solanum melongena* L. (eggplant), *Zinnia elegans* Jacq. (zinnia), and *Zea mays* L. (sweet corn).

Physical properties.—Expressed juice of young systemically infected tobacco plants was used in comparing the thermal inactivation points of the two strains and their ability to withstand dilution and

aging *in vitro*. Both were infectious after being heated 10 minutes at 70°C, and both were inactivated at 75°C. Both were infectious at dilutions of 1:10,000, but not at 1:20,000, and withstood aging *in vitro* for 4 days but not for 5 days. In dry tobacco leaves, both strains were inactive after 3 days.

Cross-protections.—When mottled leaves of zinnia plants infected with either the typical strain or the commelina strain were inoculated with Price's indicator strain (No. 6), no lesions were produced on any of 3 sets of 5 plants infected with either strain. Healthy plants inoculated with only the indicator strain developed many typical lesions. These results indicate that the virus from commelina is a strain of cucumber virus 1.

Discussion.—Fulton (2), Doolittle and Zaumeyer (1) described strains of cucumber virus 1 that consistently mottled, dwarfed, and killed plants of the resistant spinch varieties Old Dominion and Virginia Savoy. These strains are no longer available for direct comparison with the commelina strain, but the latter differs from each of them in its effects on certain hosts. Fulton's strain D, which damaged 100% of the resistant spinach plants inoculated, did not cause shriveling of the stems of infected cucumber seedlings or the V-shaped necrotic patches on old leaves of cucumber that are characteristic of the commelina strain. The latter strain also caused bright yellow vein clearing and yellow mottling of the leaves of all varieties of *N. tabacum* tested. These symptoms were not reported by Fulton. The pepper ringspot strain isolated from alfalfa by Doolittle and Zaumeyer caused severe injury to Old Dominion spinach, but differed from the commelina strain by producing ring markings on pepper leaves and fruits and failing to cause systemic infection of eggplant. It also produced primary lesions and veinal necrosis on Tepary bean, which was not infected by the commelina strain. These differences indicate that the commelina strain is not identical with either of these strains.—Plant Industry Station, Beltsville, Maryland.

LITERATURE CITED

1. Doolittle, S. P., and W. J. Zaumeyer. 1953. A pepper ringspot caused by strains of cucumber mosaic virus from pepper and alfalfa. Phytopathology 43: 333-337.
2. Fulton, J. P. 1950. Studies on strains of cucumber virus 1 from spinach. Phytopathology 40: 729-736.
3. Webb, R. E., B. A. Perry, H. A. Jones, and D. M. McLean. 1959. A new source of resistance to spinach blight. Phytopathology 50: 54-56.

HYDROLYTIC AND RESPIRATORY ENZYMES OF SPECIES OF DITYLENCHUS AND PRATYLENCHUS

L. R. Krusberg

Accepted for publication July 12, 1959.

Published with the approval of the Director as Paper No. 1017 of the Journal Series of the North Carolina Agricultural Experiment Station.

Based on a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree, North Carolina State College.

SUMMARY

Homogenates and extracts of *Ditylenchus triformis*, *D. dipsaci*, and *Pratylenchus zeae* were assayed for hydrolytic, respiratory, and terminal oxidative enzymes by techniques utilizing viscosity, titration, and colorimetric and spectrophotometric measurements.

Many enzyme systems were similar for the three nematodes. Cellulolytic (Cx) enzyme activity was present in homogenates of all three nematodes. Pectinmethylesterase and amylase activities were detected in homogenates of *D. triformis* and *D. dipsaci;* however, variable protease and no polygalacturonase activities were found in homogenates of these nematodes. Other enzymes detected in extracts from *D. triformis* and *D. dipsaci* were hexokinase, phosphoglucomutase, phosphoglyceric mutase-enolase, glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, condensing enzyme, isocitric dehydrogenase, malic dehydrogenase, "malic" enzyme, diaphorase, and DPNH-cytochrome c reductase (DPNH is reduced diphosphopyridine nucleotide). The hexokinase in extracts from *D. triformis* was active with glucose, fructose, galactose, and mannose, in order of decreasing reactivity. Lactic dehydrogenase activity was present in extracts from *D. triformis*, but was not conclusively demonstrated in extracts from *D. dipsaci*. Fumarase and cytochrome oxidase were detected in extracts from *D. triformis*. Absorption spectra of reduced versus oxidized extracts of *D. triformis* had one absorption peak around 430 mμ (Soret bands) and another much broader peak around 550 mμ (cytochrome c). Spectroscopic observations on massed intact nematodes revealed a dark absorption band around 550 mμ, and a fainter one around 605 mμ, indicating the presence of cytochromes c and $a + a_3$, respectively.

The over-all metabolism of *Ditylenchus* species seems to follow the general pattern established for the metabolism of other animals, including animal-parasitic nematodes.

Because plant-parasitic nematodes are considered obligate parasites, it is particularly difficult to study and interpret their complex relationships. Histopathological and histochemical methods have been used to investigate such interactions. Another possible approach is to study the metabolisms of the plant and parasite separately. All these methods have their limitations. Only the use of several approaches involving different techniques yields a fairly accurate

picture of host-parasite interactions.

Several recent reviews (4, 6, 8, 12) summarize our knowledge of the physiology of animal-parasitic helminths. Few investigations have dealt with the metabolism of free-living and plant-parasitic nematodes. Early studies of helminth physiology dealt principally with the hydrolysis of substrates and the effects of substrates and inhibitors on oxygen uptake with Warburg apparatus using whole or homogenized organisms. More sensitive methods and equipment, such as the spectrophotometer, have made possible more detailed and precise studies of nematode physiology.

Several hydrolytic enzymes have been studied in helminths. The presence of proteases has been demonstrated in various animal parasites (12, 28, 47). Qualitative differences in proteolytic enzymes were indicated by the varied activities of homogenates of different nematodes towards several substrates (12, 28). Protease, amylase, and invertase activities were present in distilled water in which live specimens of a *Meloidogyne* sp., *Ditylenchus destructor* Thorne, and *D. allii* (syn. *D. dipsaci* (Kühn) Filipjev) had been kept for 24 hours at 23–25°C (50). Amylase and lipase activities were found in the 2 animal parasites *Ascaris lumbricoides* L. and *Strongylus edentatus* (Looss), and the coelomic liquid of the former possessed sucrase, lactase, maltase and phosphatase activities (12). Cellulase and chitinase activities were present in homogenates of *Ditylenchus dipsaci*, *D. destructor* and *D. myceliophagus* Goodey, but not in homogenates of *Turbatrix* aceti (Müller) (48). Slight pectinase activity was detected in only one of several homogenates of *D. dipsaci* tested.

Glycolysis in helminths has probably been investigated more thoroughly than other metabolic systems, sometimes with conflicting results. A number of studies have concerned the utilization of glycolytic intermediates assayed by manometric techniques or end-product analysis using whole specimens, homogenates, and extracts of several animal parasites (4, 8, 12, 13, 26, 42). Several glycolytic enzymes have been demonstrated with spectrophotometry in extracts of *Trichinella spiralis* (Owen) larvae (14, 16). The oxygen uptake by preparations of the free-living nematode *Panagrellus silusiae* (de Man) Goodey (syn. *Anguillula silusiae*) in Warburg apparatus increased upon addition of lactate, acetate, and glycerophosphate, but in Thunberg tubes only glycerophosphate increased the rate of methylene blue decolorization (9).

Enzymes of the pentose phosphate shunt pathway were recently demonstrated in several animal parasites. Extracts of several different nematodes contained glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase activities (29). Extracts of *Ascaris lumbricoides* muscles revealed in addition to these 2 enzymes the presence of transketolase, ribose-5-phosphate dehydrogenase and xylulose-5-phosphate dehydrogenase (11).

The tricarboxylic acid cycle in nematodes has been studied by several investigators. Manometric techniques have revealed the utilization of many intermediates by animal parasites (8, 12, 14, 15). The "malic" enzyme in higher animal tissue is specifically dependent on TPN (triphosphopyridine nucleotide), but preparations from *Ascaris lumbricoides* muscles reduced DPN (diphosphopyridine nucleotide) rapidly and were one-third as active with TPN (12). Addition of glutamate, citrate, or butyrate did not increase the oxygen consumption of *Panagrellus silusiae* in Warburg apparatus, but succinate, citrate, and glutamate increased the rate of methylene blue decolorization by this nematode in Thunberg tubes (9).

A variety of chemicals have been tested for inhibition of specific enzyme systems or general respiratory metabolism of helminths. The classic competitive inhibition of succinic dehydrogenase by malonate has been demonstrated with homogenates of several animal parasites (4, 8, 12, 15) and in *Panagrellus silusiae* (9). Fluoroacetate (8), fluoride (41), trivalent antimonials (7, 33), and certain hexoses and hexose phosphates (8) have been shown to inhibit specific enzymes of helminths, but the inhibitory action of other chemicals has been less defined (4, 8, 9, 26).

The cytochromes and hemoglobins have been searched for and studied in several helminths (4, 8, 12). Cytochromes a, b, and c were observed spectroscopically in whole, packed larvae of *Trichinella Spiralis* and extracts possessed the activity of 3 enzymes active in terminal oxidation (17). There is also evidence that cytochromes are present in *Panagrellus silusiae* (9). Cytochromes are lacking in some animal parasites (8) and there is evidence that FAD (flavinadenine dinucleotide) is important in their terminal oxidation mechanisms (4, 12).

The present investigations were made to determine, through the detection of specific hydrolytic and respiratory enzymes, if the metabolism of free-living and plant-parasitic nematodes was similar to that of animal-parasitic nematodes and higher animals. The specific objectives were: 1) to develop methods by which the enzyme activities of nematodes could be demonstrated, 2) to demonstrate the activity of nematode enzymes that involve hydrolysis of host components, and 3) to demonstrate the activity of nematode respiratory enzymes.

The two nematode species principally used in these studies, *Ditylenchus triformis* Hirschmann and Sasser and *D. dipsaci*, were quantities needed to prepare homogenates and extracts. *Pratylenchus zeae* Graham was used in a few tests.

Materials and methods.—*Sources of nematodes.*—The bisexual population of *Ditylenchus triformis* (obtained from Dr. Hedwig Hirschmann) used was propagated on a *Fusarium*-like fungus growing on PDA (potato-dextrose agar) in Petri plates at 23–30°C (19). Nematode reproduction was abundant 2–3 months after the cultures were initiated. A constant supply of nematodes was maintained by periodically transferring a plug of PDA containing the fungus and nematodes from an old culture to fresh plates of medium. Nematodes were separated from the medium

and fungus by placing the whole culture on wet-strength tissue paper on plastic screens in Baermann funnels kept at room temperature. After 24 hours, nematodes were collected from the funnels in a small amount of water. Eighty 9-cm Petri plates supplied enough nematodes for an extract or homogenate.

D. dipsaci specimens were obtained from infected alfalfa hay collected (June, 1958) from a naturally infested field, dried indoors, and stored in bags at room temperature. A small amount of 2% Chlordane was applied to the bags to keep insects out. For extraction of nematodes the hay was placed on wet-strength tissue paper on a plastic screen fastened to a 1.5×4-ft. wooden frame tilted about 15° from the horizontal, and a continuous spray of water mist was applied. A plastic container under the lower edge of the screen caught the water dripping off. The distance between the point where the water dripped into the container and the overflow was sufficient for the nematodes to settle to the bottom and not be washed out with the overflow water. The hay was usually sprayed with mist for 36 hours at room temperature. The water suspension of nematodes was placed in plastic buckets at 17°C and the nematodes were allowed to settle out. The volume of the nematode suspension was reduced to about 100 ml by repeating the settling process twice more. Nematodes from 3 or 4 batches of hay were generally sufficient for a series of tests.

Specimens of *Pratylenchus zeae* were obtained from infected roots of N.C. 27 field corn growing in the greenhouse in 6-in. pots. Nematode cultures were established by mixing a small quantity of finely chopped infected roots with methyl bromide-treated soil, placing the soil in pots, and planting corn seed. Fertilizer applied was sufficient for good plant growth. At 12–14 weeks, when the corn plants became senescent, some of the roots were discolored and numbers of *P. zeae* maximum. Other nematode species present amounted to less than 5%. The infected corn roots were thoroughly washed and chopped to small pieces with scissors. The nematodes were collected by the technique described for *D. dipsaci*.

Preparation of nematode homogenates and extracts.—The concentrated suspensions of nematodes were pelleted by centrifugation and the supernatant removed with a pipette. To eliminate most bacteria and soluble substances present in the suspensions the nematodes were resuspended and centrifuged a few seconds at 680 G, 8 times with distilled water and twice with buffer or sucrose solution. After each washing the supernatant was discarded and the process repeated with fresh solution. These massed nematodes were ground to prepare homogenates or extracts, or were placed at 4° C until used.

Nematode homogenates for the assay of hydrolytic enzymes were prepared as follows: 1–5 ml of the washed, packed nematodes in 0.25*M* sucrose or 0.1*M* potassium phosphate buffer, pH 7.4, were placed in a 15-ml Ten-Broeck ground-glass tissue grinder held in an ice bath. About 10 mg of glutathione were added to the nematodes and they were homogenized for 10–20 minutes. Cold sucrose or phosphate buffer solution was added to the homogenate to make a final volume of 8–10 ml. About 3 ml of this homogenate was removed to a test tube and heated 5–10 minutes in a boiling water bath to prepare the boiled homogenate. Sucrose solution was added to homogenates for cellulolytic (Cx), polygalacturonase, pectin-methylesterase, and proteolytic enzyme assays. Phosphate buffer was added to homogenates for amylase assays. Protein content of the homogenates was assayed colorimetrically after the method of Lowery et al (30) at 650 mμ against a casein standard calibration curve. Protein contents of the different homogenates varied from 2.9 to 12.5 mg of protein per ml, with most about 10 mg.

Nematode extracts for the assay of other enzymes, except cytochrome oxidase, were prepared as follows: 3–6 ml of the washed, packed nematodes in buffer were added directly to 1–5 g of ground glass (200-mesh, Fisher) in a small cold mortar. In some cases the nematodes were further concentrated by filtration with a medium-porosity sintered-glass filter before being placed in the mortar. In the first extracts about 10 mg of glutathione was added to the mixture before grinding, to complex any enzyme-inhibiting heavy metals possibly present. This was omitted later because it interfered with certain assays. The mortar was placed in ice and removed to a cold room at 3°C, where the nematodes were ground for 10–20 minutes. Sometimes more ground glass or buffer was added to obtain a thick paste of the nematodes and glass during the grinding; this greatly helped maceration of the nematodes. Microscopic examination revealed that nearly all the nematodes, except for a few larvae, were consistently macerated in this way. Several ml of cold buffer were added to the ground nematodes, depending on the quantity of extracts desired (usually 3–5 ml), and the mixture was stirred in the cold for 10 minutes to extract the enzymes from the macerated nematodes. The slurry was transferred to 7-ml plastic centrifuge tubes and kept in an ice bath until centrifuged at 2°C for 15 minutes at 25,000 G. The clear yellowish or colorless supernatant was used for the enzyme assays. A portion of this extract was placed in a test tube in a boiling water bath for 5 minutes to prepare the boiled extract. Protein content of the extracts was measured spectrophotometrically by 260–280 mμ absorption (21). Protein contents of the different extracts varied from 1.9 to 9.4 mg of protein per ml, averaging about 3 mg. Extracts were not diluted to a constant protein concentration. Most were prepared with 0.1*M* potassium phosphate buffer at pH 7.4, but some, including all where the enzyme assay procedure called for manganous ions, were prepared with 0.05*M* glycylglycine buffer at pH 7.4.

All the extracts for the assay of DPNH-cytochrome c reductase (DPNH is reduced DPN) and some for diaphorase were dialyzed to remove reducing compounds. The extract (2–3 ml) was placed in a Cellophane dialysis sac and suspended in 1 liter of 0.1*M*

potassium phosphate buffer, pH 7.4, surrounded by ice. The buffer was stirred to speed dialysis, and the whole apparatus was kept in a cold room at 3°C. The extracts were dialyzed 16–18 hours.

Nematode extracts for spectral analyses for cytochrome pigments and cytochrome oxidase were prepared as follows: about 5 ml of packed, washed nematodes were homogenized 20 minutes in a 15-ml ground-glass tissue grinder kept in ice. Usually about 95% of the nematodes were broken. Then 2% digitonin or 20% sodium cholate was added, making respective final concentrations of 1% and 2% in the homogenate, and slight additional homogenization mixed in the surface-active agent. The pH of the homogenates was adjusted to and maintained at about 8.0 with KOH. The homogenates were incubated about 4 hours at room temperature (21–24°C) and centrifuged 15–30 minutes at 25–50,000 G at 2°C. The clear greenish-yellow supernatant solutions were diluted to 6 ml and placed in 3-ml 1.0-cm quartz cuvettes. To reduce any cytochrome pigments, a small amount of sodium hydrosulfite was added to one cuvette, and an oxidized-versus-reduced spectrum was made from 400 to 625 mμ at 1-mμ intervals. One such sodium cholate extract was used for cytochrome oxidase assay.

The spectroscopic examination of intact living nematodes for cytochromes utilized packed nematodes that had been washed with distilled water. About 3 ml of nematodes were placed in a 5-ml beaker and observed directly under the spectroscope before and after being mixed with a few mg of sodium hydrosulfite. The nematode spectrum was compared with that of a suspension of Baker's yeast similarly treated.

Assay systems for hydrolytic enzymes.—Cellulolytic (Cx) enzymes and polygalacturonase were assayed by viscometric methods (27). Solutions of 1.2% carboxymethylcellulose (Cellulose gum, Type 7MP, Hercules Powder Company) and 1.2% sodium polypectate (California Fruit Growers Exchange) in distilled water were prepared as substrates, and a small amount of toluene was added to inhibit bacterial activity. Ostwald-Fenske No. 300 viscosity pipettes were supported in a constant-temperature bath at 29±0.5°C. 5 ml of substrate were added, and 10 minutes was allowed for the tubes to come to temperature equilibrium. Then 1 ml of nematode homogenate or sucrose solution was added to a tube and mixed, and a time-flow measurement was made immediately. Thereafter, time-flow measurements on each tube were made at 10- or 20-minute intervals for the first hour, and 20-minute intervals for the second hour. For each assay and each substrate, 1 pipette contained homogenate, a second boiled homogenate, and a third sucrose solution. Three different homogenates were tested for each of the 2 *Ditylenchus* species, but *P. zeae* nematodes were sufficient for only a single test. The data were analyzed according to the formula:

$$\frac{A-B}{A-W} \times 100 = \% \text{ loss in viscosity}$$

where A = initial flow time, B = flow time after an interval, and W = flow time of water. The pH of the mixtures after completing the assays varied from 6.2 to 6.8.

The mixtures from the Cx assays after viscometric measurements were tested with Benedict's solution for the presence of reducing groups. One ml of the assay mixture was mixed with 5 ml of Benedict's solution in a test tube and heated 5 minutes in a boiling water bath. A change from blue to green or a precipitate of copper oxide indicated a positive test.

Pectinmethylesterase activity was measured after the method of Smith (45). A solution of 0.001*M*

Fig. 1. Enzyme activities of nematode homogenates and extracts. **A)** Hydrolysis of a 1.2% carboxymethylcellulose solution by homogenates of *D. triformis*, *D. dipsaci*, and *P. zeae*. Protein contents were in mg per ml of homogenates for *D. dipsaci*, 6.7, and *P. zeae*, 2.9; *D. triformis* was not measured. **B)** Hexokinase activity in extracts of *D. triformis* and *D. dipsaci* (coupled to glucose-6-phosphate dehydrogenase). The assay medium (3.0 ml) contained 60 μM tris-HCl buffer at pH 7.5; 100 μM $MgCl_2 \cdot 6H_2O$; 100 μM ATP neutralized with KOH; 8 μM of one of the hexoses, glucose, fructose, galactose or mannose; 0.15 M TPN; and 0.1 ml of extract or boiled extract. The complete medium contained all the above components. The controls were the complete system lacking hexose, lacking ATP or with boiled extract. Controls of the complete minus ATP were made for each hexose investigated. Because of the large number of values, only the curves for the limits of the controls were drawn in this figure. The *D. triformis* extract contained 6.9 mg protein per ml, and the *D. dipsaci* extract 3.9 mg of protein per ml. **C)** Phosphoglucomutase activity in extracts from *D. triformis* and *D. dipsaci* (coupled to glucose-6-phosphate dehydrogenase). The assay medium (3.0 ml) contained 60 μM of tris-HCl buffer at pH 7.5; 20 μM of $MgCl_2 \cdot 6H_2O$; 8 μM of potassium glucose-1-phosphate; 0.15 μM of TPN; 16.6 μM of cysteine HCl neutralized with KOH; and 0.1 ml of extract or boiled extract. The complete medium contained all the above components, and the controls lacked glucose-1-phosphate or contained boiled extract. The *D. triformis* extract contained 6.6 mg protein per ml, and the *D. dipsaci* extract 3.9 mg per ml. **D)** Phosphoglyceric mutase-enolase activity of extracts from *D. triformis* and *D. dipsaci*. The assay medium (3.0 ml) contained 66 μM potassium phosphate buffer at pH 7.4; 15 μM $MgCl_2 \cdot 6H_2O$; 20 μM potassium 3-phosphoglycerate; 0.1 ml extract or boiled extract; and 20 μM sodium fluoride. The complete assay medium contained all the above components except the sodium fluoride, which was added as an inhibitor after 15–30 minutes of incubation. Controls consisted of the complete medium minus potassium 3-phosphoglycerate and the complete medium with boiled extract. The *D. dipsaci* extract contained 5.2 mg protein per ml; protein was not measured in the *D. triformis* extract. **E)** Lactic dehydrogenase activity in an extract from *D. triformis*. The assay medium (3.0 ml) contained 100 μM potassium phosphate buffer at pH 7.4; 1 μM sodium pyruvate; 1 μM NaCl; 0.2 μM DPNH; and 0.2 ml extract. The complete assay medium contained all the above components; the controls lacked sodium pyruvate. The extract contained 9.0 mg protein per ml. **F)** Glucose-6-phosphate dehydrogenase activity in extracts from *D. triformis* and *D. dipsaci*. The asasy medium (3.0 ml) contained 10 μM glycylglycine buffer at pH 7.5; 4 μM potassium glucose-6-phosphate; 20 μM $MgCl \cdot 6H_2O$; 0.15 M TPN; and 0.1 ml extract. The complete system contained all the above components; the controls lacked potassium glucose-6-phosphate. The *D. triformis* extract contained 2.6 mg protein per ml, and the *D. dipsaci* extract 5.2 mg per ml.

NaOH was used to titrate back to the original color.

Protease activity was measured by the method of Herriot (18), modified as follows: substrate was a solution of 2% hemoglobin in distilled water; color density was measured in a photoelectric colorimeter at 650 mμ; and the readings were plotted against a

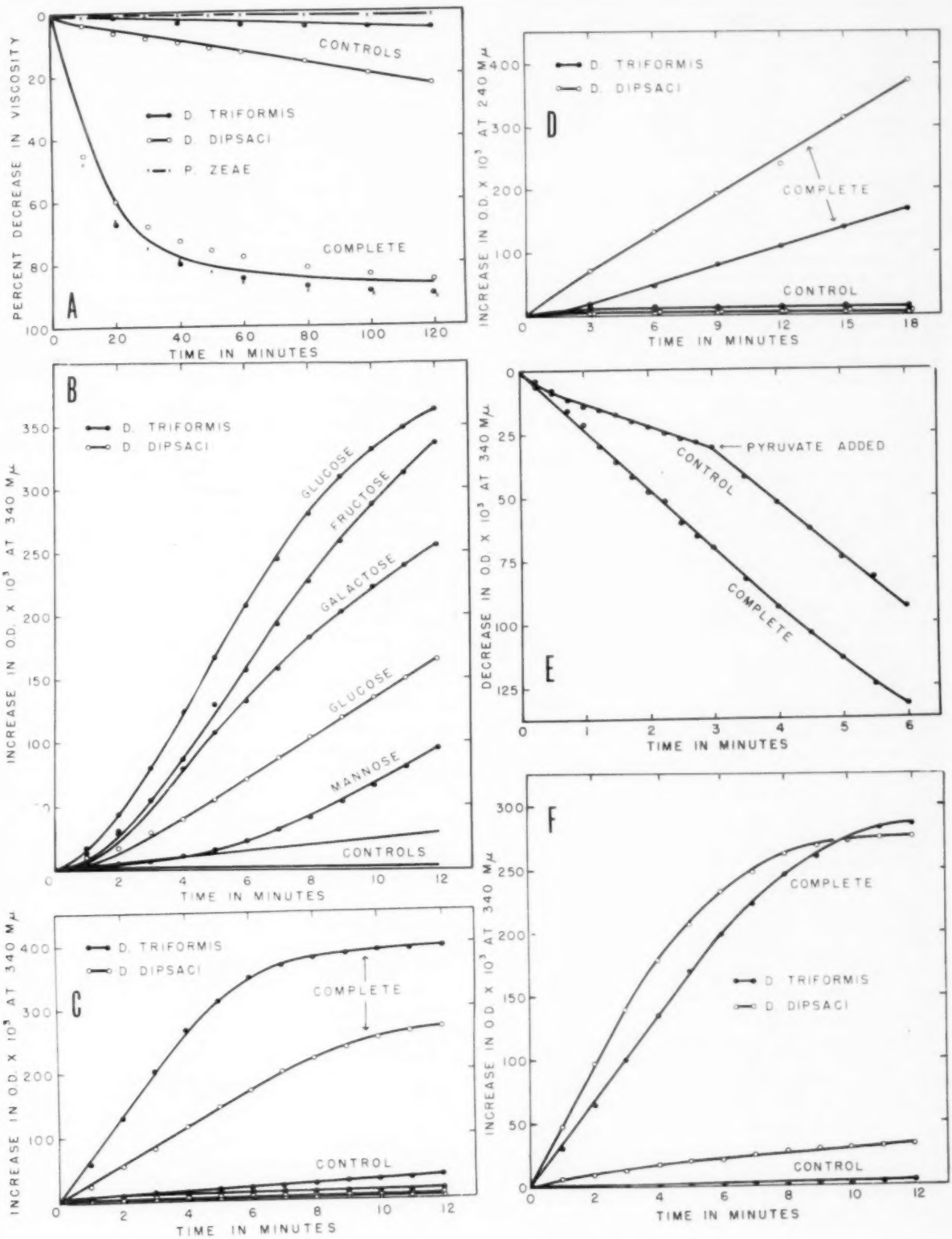


TABLE 1.—*Pectinmethylesterase activity of homogenates of D. triformis and D. dipsaci*[a]

Homogenate number	Protein (mg/ml)	Hours incubated	μM of NaOH added to neutralize acid formed		
			Homogenate	Boiled homogenate	0.25*M* sucrose
D. triformis					
1	–	11	4.6	2.7	1.0
2	–	21	12.3	7.9	2.0
3	–	29	27.6	16.6	2.9
4	–	40	13.9	7.6	0.2
D. dipsaci					
1	9	60	39.1	28.5	6.8
2	12	48	10.2	5.2	3.3

[a]Substrate composition per 100 ml distilled water solution: 0.5 g pectin, 0.2 g phenol, 0.58 g NaCl, and 2.0 ml 0.1% bromothymol blue. 7.4 ml of substrate mixed with 0.6 ml of homogenate, boiled homogenate, or 0.25*M* sucrose were adjusted to a blue color with 1*N* NaOH, and the tubes then incubated at 30°C. Tubes were titrated to the original color with 0.001*M* NaOH at the end of the incubation period.

tyrosine calibration curve.

Amylase activity was assayed, using 3,5-dinitrosalicylic acid to measure the reducing groups (1). Readings were made on a photoelectric colorimeter at 525 mμ and plotted against a maltose calibration curve.

Assay systems for respiratory enzymes.—Hexokinase and phosphoglucomutase activities were assayed by coupling with the glucose-6-phosphate dehydrogenase naturally present in the nematode extracts and measuring the rate of reduction of TPN spectrophotometrically at 340 mμ (40). This reaction was dependent upon the formation of glucose-6-phosphate from the substrate added. Glucose-6-phosphate dehydrogenase (25), 6-phosphogluconic dehydrogenase (20), isocitric dehydrogenase (39) and "malic" enzyme (37) activities were measured spectrophotometrically at 340 mμ by the rate of TPN reduction. Lactic dehydrogenase (24) and malic dehydrogenase (36) activities were assayed by measuring the rate of oxidation of DPNH spectrophotometrically at 340 mμ. Alcohol dehydrogenase (3), glucose dehydrogenase (46), condensing enzyme (38) and α-ketoglutaric dehydrogenase (22) activities were assayed by measuring the rate of DPN reduction spectrophotometrically at 340 mμ. Enolase activity was assayed by measuring the absorption of phosphoenolpyruvic acid spectrophotometrically at 240 mμ as it was formed (5). Actually the system involved a coupled reaction necessitating the presence of phosphoglyceric mutase in the extracts, since the substrate, 3-phosphoglyceric acid, had to be transformed by this latter enzyme to 2-phosphoglyceric acid, which is the substrate for enolase. Fumarase activity was measured spectrophotometrically by the decrease in optical density at 300 mμ as fumarate was hydrated to malate (34). Succinic dehydrogenase activity was measured using the reduction of potassium ferricyanide spectrophotometrically at 400 mμ (2). DPNH-cytochrome c reductase and diaphorase (31, 32) activities were respectively measured spectrophotometrically by the rate of reduction of ferricytochrome c at 550 mμ and 2,6-dichlorophenolindophenol at 600 mμ. Cytochrome oxidase activity was measured spectrophotometrically simply by the rate of oxidation of ferrocytochrome c (supplied by Dr. H. J. Evans) at 550 mμ (44). The enzyme assay systems are presented in the legends to the figures.

The colorimetric measurements were made with a Fisher Electrophotometer, and the spectrophotometric assays with a Beckman DU Spectrophotometer. An error is involved in the spectrophotometric measurements of activity against time in the first 15-second interval. A period of 8–10 seconds elapsed between the mixing of reagents in the cuvettes and the zero-time reading, so the 15-second reading is actually based on only a 5–7-second interval although the curves are drawn to zero time. All assays, except those involving viscosity and pectinmethylesterase measurements, were made at room temperature, which varied from 21 to 26°C.

In most cases the data are based on assays of at least 3 different homogenates or extracts of any 1 nematode species. With *P. zeae*, only enough nematodes were available for a single assay. In a few cases only 2 assays were made of a particular system. Only one assay of cytochrome oxidase was performed, for the supply of ferrocytochrome c was limited. The data for a given enzyme system were highly consistent from 1 assay to another, so only 1 assay for each nematode species for each enzyme is illustrated in the experimental results.

Experimental results.—*Hydrolytic enzymes.*—The cellulolytic (Cx) activity of homogenates of *D. triformis*, *D. dipsaci*, and *P. zeae* are illustrated in Fig. 1-A. Homogenates of all 3 nematodes caused a fairly

TABLE 2.—*Proteolytic activity of homogenates of D. triformis and D. dipsaci*[a]

Homogenate number	Protein (mg/ml)	Hours incubated	Equivalent in mg of tyrosine per ml		
			Homogenate	Boiled homogenate	0.25*M* sucrose
D. triformis					
1	–	0.5	0.65	0.61	0.15
2	–	2	0.66	0.61	0.24
3	–	6	0.42	0.41	0.25
4	–	24	0.84	0.67	0.30
5	2.4	2	0.65	0.63	0.23
6	1.1	2	0.46	0.44	0.17
7	14.0	1	0.75	0.74	0.23
D. dipsaci					
1	9.0	1	0.96	0.82	0.22
2	12.5	1	1.03	0.84	0.28
3	6.7	1	0.83	0.82	0.23
4	1.7	6	0.51	0.45	0.20

[a] 5.0 ml of aqueous 2% hemoglobin (pH 7.5) was incubated with 1 ml of the homogenate, boiled homogenate, or sucrose solution at 21–25°C. Toluene was added to the substrate to inhibit bacterial activity.

TABLE 3.—*Amylase activity of homogenates of D. triformis and D. dipsaci*[a]

Homogenate number	Protein (mg/ml)	Hours incubated	Equivalent mg of maltose formed				
			Starch + homogenate	Starch alone	Homogenate alone	Starch + boiled homogenate	Water blank
D. triformis							
1	–	0.5	.120	.042	.068		.007
2	–	4	1.02	.037	.022	.058	.008
3	–	1	.108	.038		.062	
4	2.4	2	.850	.041	.029	.084	.008
5	1.1	2	.640	.043	.016	.058	.009
D. dipsaci							
1	3.1	2	.112	.072	.007	.073	.006
2	4.1	2	.131	.077	.006	.078	.007

[a] Substrate: 1% soluble starch in 0.02*M* potassium phosphate buffer at pH 6.9 containing 0.0067*M* NaCl. One ml of substrate was incubated with 1-ml aliquots of the other components to make a total volume of 2.0 ml in each tube. Dilutions were made with distilled water. Toluene was added to the mixture to inhibit bacterial activity. Incubated at 21–24°C.

rapid decrease in viscosity of carboxymethylcellulose solutions. Since the activity values of the enzymes in homogenates of the 3 nematodes were so similar, only a single curve was drawn to represent these active systems. Homogenates of *D. triformis* and *P. zeae* boiled for 5 minutes showed very slight or no enzyme activity; those of *D. dipsaci* boiled for 5 minutes caused about a 40% loss in viscosity in 2 hours, but when boiled 10 minutes cause a loss of about 20% in 2 hours. The curves designated "controls" in Fig. 1-A present the loss in viscosity in pipettes to which boiled homogenate was added. The sucrose blanks with no homogenates showed very little change in viscosity, and are not shown. The pH values of the reaction mixtures were around 6.4.

After viscometric measurements were made of Cx activity, 1 ml of the reaction mixture in each tube was tested with Benedict's solution for the presence of reducing groups. The mixtures with the active homogenates gave very strong positive reactions; equivalent amounts of homogenates alone gave slight positive reactions; the mixtures with boiled homogenates gave very faint positive reactions; and the sucrose controls without homogenates were negative.

No hydrolysis of sodium polypectate could be detected viscometrically with homogenates of *D. triformis* or *D. dipsaci*. A check with 1 ml of a 100-ppm Pectinol-100D (Rohm and Haas) solution indicated that the added toluene was not affecting hydrolysis of the substrate. A very small amount of polygalacturonase activity appeared to be present in the 1 homogenate of *P. zeae*, but its presence remains questionable until more positive results are obtained. The pH values of the reaction mixtures were around 6.3.

Pectinmethylesterase activity was present in homogenates of both *D. triformis* and *D. dipsaci* (Table 1). Boiling a homogenate 5 minutes reduced the amount of hydrolysis of pectin to methanol and polygalacturonic acid but, even with boiled homogenates, there was more ester hydrolysis than in the controls, where sucrose solution was substituted for homogenate. It was often possible to note the difference in pH in the tubes after 3 hours of incubation by a color change by bromothymol blue from blue to yellow.

Definite proteolytic activity was not observed in any of the homogenates of *D. triformis* tested; however, 2 of 4 homogenates of *D. dipsaci* had activity (Table 2). Although, in every case, amino acid content was lower with boiled homogenates of *D. triformis* incubated with hemoglobin solution than with unboiled homogenates based on a tyrosine calibration curve, the differences fell within the experimental error of the method (22).

Amylase activity was present in homogenates of both *D. triformis* and *D. dipsaci* (Table 3). Boiling the homogenate for 5 minutes destroyed all enzyme activity.

Respiratory enzymes.—1) *Enzymes of glycolysis.*—Hexokinase was assayed by coupling with the glucose-6-phosphate dehydrogenase endogenous in the extracts. Extracts of both *D. triformis* and *D. dipsaci* possessed very active hexokinases (Fig. 1-B). Only phosphorylation of glucose was tested with extracts of *D. dipsaci*, but glucose, fructose, galactose, and mannose were tested with extracts of *D. triformis*. In the absence of one of the above sugars or ATP (adenosine triphosphate) there was slight reduction of TPN. This was probably caused by substances in the crude extract able to reduce TPN. With boiled extract plus glucose and ATP, or glucose plus ATP without extract, there was no reduction of TPN. All the controls—complete with boiled extract, complete minus ATP, or complete minus hexose—fell within the range indicated in Fig. 1-B. All the hexoses were in equimolar concentration, and the descending order of reactivity was glucose, fructose, galactose, and mannose. With mannose there was a lag of 4–5 minutes at the beginning of the assays before the rate of TPN reduction became pronounced. There was also a slight lag (about 1 minute) with the other 3 sugars.

Phosphoglucomutase activity of the nematode extracts was also assayed by coupling to the glucose-6-phosphate dehydrogenase demonstrated in the extracts. Phosphoglucomutase activity was present in extracts

of both *D. triformis* and *D. dipsaci* (Fig. 1-C). As in the assay for hexokinase, there was a slight reduction of TPN in the absence of the substrate, glucose-1-phosphate. Boiling the extracts for 5 minutes destroyed almost all the ability to reduce TPN. Assay systems lacking substrate or with boiled extract served as the controls. In most of the assays the reaction rate as measured by the rate of TPN reduction was linear for the first 4–5 minutes and then gradually leveled off. In 2 assays in which lower concentrations of enzymes were added the reaction rates were linear for 10 and 12 minutes.

Phosphoglyceric mutase-enolase activity was present in extracts of both *D. triformis* and *D. dipsaci* (Fig. 1-D). Boiled extract or absence of the substrate in the assay systems prevented any increase in OD (optical density), as illustrated in the controls. The addition of sodium fluoride to the active system immediately stopped formation of phosphoenolpyruvic acid as evidenced by a leveling off of the curve. In most assays the reaction rate was linear throughout the test period of 15–24 minutes. In 2 assays with *D. dipsaci* extracts there was a slight lag for the first several minutes before the reaction rates became linear.

Lactic dehydrogenase activity was present in extracts of *D. triformis* (Fig. 1-E), but activity was only slight in 1 extract and absent in 2 additional extracts of *D. dipsaci*. Sodium malonate (10 μM) inhibited enzyme activity slightly, and sodium iodoacetate (10 μM) even more, in extracts of *D. triformis*. In the control cuvettes lacking pyruvate there was some oxidation of DPNH, but when pyruvate was added the activity curve immediately shifted to a slope parallel with the complete system. Because of the low concentrations of enzyme the reaction rates were almost linear over the entire assay period of 6 minutes.

No alcohol or glucose dehydrogenase activities were detected in extracts of *D. triformis*. The assay medium in a final volume of 3.3 ml for alcohol dehydrogenase contained 300 μM of glycine-NaOH buffer at pH 9.6, 1 μM of DPN, 0.1 ml of 95% ethanol, and 0.1 ml of extract. The assay medium for glucose dehydrogenase in a final volume of 3.0 ml contained 130 μM of potassium phosphate buffer at pH 7.6, 200 μM of glucose, 0.15 μM of DPN, and 0.1 ml of extract.

2) *Enzymes of the pentose phosphate shunt pathway.*—Both glucose-6-phosphate (Fig. 1-F) and 6-phosphogluconic (Fig. 2-A) dehydrogenases were present in extracts of *D. triformis* and *D. dipsaci*. There was some reduction of TPN in the absence of added substrates, as with the hexokinase assay. The curves designated controls represent assay systems from which the substrates were omitted. The reaction rates were always linear for the first 4–6 minutes, but with lower enzyme concentrations were linear for the entire 12 minutes. When glucose-6-phosphate was added to the control cuvette after 7 minutes of incubation the resulting reaction rate was more rapid than in the cuvette that contained all components at the beginning of the assay.

3) *Enzymes of the tricarboxylic acid cycle.*—Condensing enzyme activity was present in extracts from *D. triformis* and *D. dipsaci* (Fig. 2-B). This assay system was coupled with malic dehydrogenase. Since extracts of both nematodes had very active malic dehydrogenase activities no purified enzyme was added to the reaction mixture. All the components of the reaction mixture except acetyl-CoA (acetyl-Coenzyme A) were mixed and the reaction allowed to come to equilibrium (in about 2 minutes). Then acetyl-CoA was added to the mixture and the rate of DPN reduction measured. The over-all reaction is:

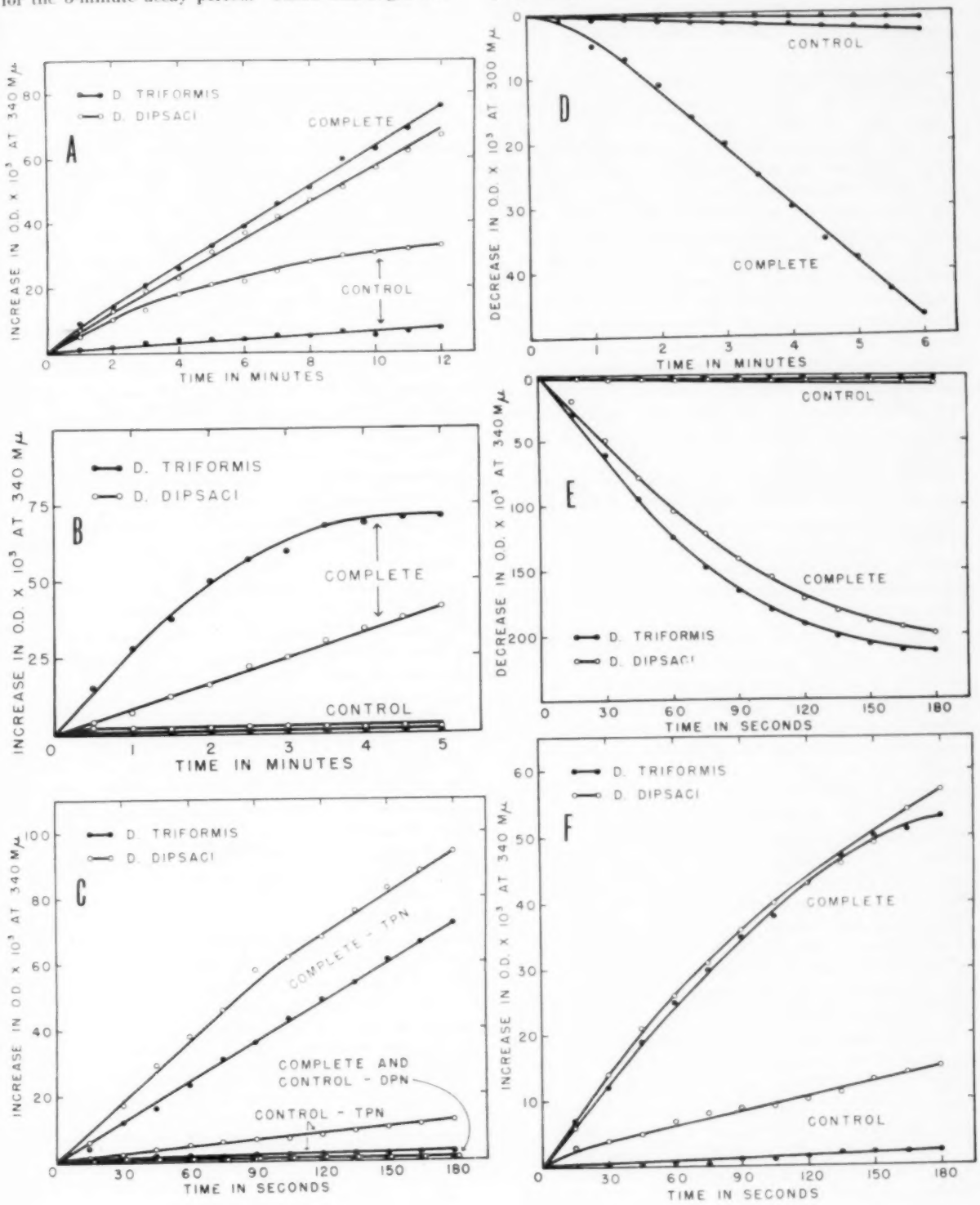
$$\text{Acetyl-CoA} + \text{1-malate}^{=} + \text{DPN}^{+} + \text{H}_2\text{O} \rightleftharpoons \text{citrate}^{\equiv} + \text{CoA} + \text{DPNH} + 2\text{H}^{+}$$

Only in the complete system was there condensing enzyme activity. In the absence of 1-malate there was no reduction of DPN (control). One assay of a *P. zeae* extract indicated the presence of this enzyme in that nematode also. DPN reduction was rapid, and usually linear for the first minute or 2 and then leveled

Fig. 2. Enzyme activities of nematode extracts. **A)** 6-phosphogluconic dehydrogenase activity in extracts from *D. triformis* and *D. dipsaci*. The assay medium (3.0 ml) contained in 10 μM glycylglycine buffer at pH 7.5; 4 μM sodium 6-phosphogluconate; 20 μM $MgCl_2 \cdot 6H_2O$; 0.15 μM TPN; and 0.1 ml extract. The complete system contained all the above components; the control was similar, but lacked sodium 6-phosphogluconate. The *D. triformis* extract contained 3.3 mg protein per ml, and the *D. dipsaci* extract 5.2 mg per ml. **B)** Condensing enzyme activity in extracts from *D. triformis* and *D. dipsaci*. The assay medium (3.0 ml) contained 150 μM tris-HCl buffer at pH 8.0; 20 μM potassium 1-malate; 0.6 μM DPN; 0.4 μM acetyl-coenzyme A; and 0.1 ml extract. The complete system contained all the above components; the control was similar, but lacked potassium 1-malate. The *D. triformis* extract contained 13.4 mg protein per ml, and the *D. dipsaci* extract 1.7 mg per ml. **C)** Isocitric dehydrogenase activity in extracts from *D. triformis* and *D. dipsaci*. The assay medium (3.0 ml) contained 75 μM glycylglycine buffer at pH 7.4; 0.6 μM sodium or potassium dl-isocitrate; 0.13 μM TPN or 0.15 μM DPN; 1.8 μM $MnCl_2 \cdot 4H_2O$; and 0.1 ml extract. The complete system contained all the above components; the control was similar, but lacked dl-isocitrate. The *D. triformis* extract contained 2.6 mg protein per ml, and the *D. dipsaci* extract 5.2 mg per ml. **D)** Fumarase activity in an extract from *D. triformis*. The assay medium (3.0 ml) contained 17 μM sodium fumarate; 92 μM potassium phosphate buffer at pH 7.3; and 0.1 ml extract or boiled extract. The complete system contained all the above components; the controls were similar, but lacked sodium fumarate or contained boiled extract. The protein content of this extract was 9.4 mg per ml. **E)** Malic dehydrogenase activity in extracts from *D. triformis* and *D. dipsaci*. The assay medium (3.0 ml) contained 75 μM glycylglycine buffer at pH 7.4; 0.76 μM potassium oxalacetate; 0.15 μM DPNH; and 0.1 ml 1:10 distilled-water-diluted extract. The complete system contained all the above components; the control was similar, but lacked potassium oxalacetate. The *D. triformis* extract contained 13.4 mg protein per ml, and the *D. dipsaci* extract 12.5 mg per ml. **F)** "Malic" enzyme activity in extracts from *D. triformis* and *D. dipsaci*. The assay medium (3.0 ml) contained 75 μM glycylglycine buffer at pH 7.4; 1.5 μM potassium 1-malate; 0.14 μM TPN; 3 μM $MnCl_2 \cdot 4H_2O$; and 0.1 ml extract. The complete system contained all the above components; the control was similar, but lacked potassium 1-malate. The *D. triformis* extract contained 6.6 mg protein per ml, and the *D. dipsaci* extract 5.2 mg per ml.

off. With a dilute extract, activity was linear for 6 minutes.

Isocitric dehydrogenase activity was present in extracts of both *D. triformis* and *D. dipsaci* (Fig. 2-C). In all the assays the rate of TPN reduction was linear for the 3-minute assay period. There was slight TPN reduction in the absence of added substrate, as seen in the control curves. However, when isocitrate was added to the control cuvette, activity paralleled that in the complete system. When DPN was substituted for TPN there was no activity in any combination (Fig. 2-C).



Fumarase activity was present in extracts of *D. triformis* (Fig. 2-D). Lack of nematodes made it impossible to check *D. dipsaci* extracts for this enzyme. In this reaction there is a decrease in OD at 300 mμ as the fumarate is transformed to malate. In the control cuvette there was no activity until fumarate was added, and then the curve paralleled that of the complete system. Boiled extract lacked enzyme activity.

Malic dehydrogenase was the most active enzyme found in the extracts of *D. triformis* and *D. dipsaci* (Fig. 2-E). In almost every case it was necessary to dilute the extracts 1:10 with distilled water to be able to measure the rate of DPNH oxidation. In the absence of substrate there was no oxidation of DPNH, but when substrate was added to the control cuvette after some seconds' incubation, a sharp bend occurred in the curve and DPNH oxidation proceeded at a rate parallel to that of the complete system. From a single assay it was found that an extract of *P. zeae* also contined this enzyme. It was noted that sodium fumarate (17 μM), sodium malonate (10 μM) and sodium iodoacetate (10 μM) inhibited malic dehydrogenase activity only slightly in extracts of *D. triformis*, and sodium fumarate (85 μM) also produced only slight inhibition of this enzyme in extracts of *D. dipsaci*.

"Malic" enzyme activity was present in extracts of both *D. triformis* and *D. dipsaci* (Fig. 2-F). In the absence of substrate there was little or no reduction of TPN. The activity curves were usually linear for the assay periods of 3 minutes. Addition of malate to the control cuvette immediately increased the rate of TPN reduction.

With potassium ferricyanide as the electron acceptor it was not possible to obtain clear-cut evidence of the presence of succinic dehydrogenase activity in extracts of *D. triformis* or *D. dipsaci* spectrophotometrically. There appeared to be activity in a few extracts, but in others the ferricyanide was reduced as rapidly in the absence of succinate as in its presence. The assay system in a total volume of 3.0 ml contained 30 μM of KCN, 3 μM of potassium ferricyanide, 40 μM of sodium succinate, 300 μM of potassium phosphate buffer at pH 7.2, and 0.1–0.2 ml of extract. In addition, α-ketoglutaric dehydrogenase activity was not detected in extracts of either nematode. The assay system in a total volume of 3.0 ml contained 0.13 μM of Coenzyme A, 100 μM of potassium phosphate buffer at pH 7.4, 0.27 μM of DPN, 8.3 μM of cysteine neutralized with KOH, 25 μM of potassium α-ketoglutarate, and 0.1–0.2 ml of nematode extract.

Electron transport systems.—Diaphorase activity, or the transfer of electrons from DPNH to 2,6-dichlorophenolindophenol, was found in extracts of both species of *Ditylenchus* (Fig. 3-A). In the controls there was some reduction of the dye by the extracts in the absence of DPNH, indicating the presence of reducing substances in the extracts. A similar amount of chemical reduction of the dye occurred in the complete system lacking extract. Boiled extract caused slightly more rapid dye reduction than either the complete system minus DPNH or the complete system minus extract, but was far below the rate of reduction of the complete system with unboiled extracts. The control cuvettes containing extracts of *D. dipsaci* had much lower rates of dye reduction than those containing extracts of *D. triformis*, making more striking the difference between the complete and the control curves. When extracts of *D. triformis* were dialyzed and tested, enzyme activity was reduced. However, in one extract there was complete dependence on the presence of DPNH to obtain reduction of the dye.

DPNH-cytochrome c reductase activity was present in extracts of *D. triformis* and *D. dipsaci* (Fig. 3-B). A DPNH stimulus of the rate of ferricytochrome c reduction could be demonstrated only when the extracts had been dialyzed 16–18 hours, and then the stimulus was not great. A complicating factor in the assays was a turbidity in the reaction mixtures apparently due to a complex formed between the protein of the extracts and the ferricytochrome c. The presence or absence of DPNH did not appear to affect the amount of turbidity. The control curves are designated "minus DPNH" and "minus extract" in Fig. 3-B.

Cytochrome oxidase activity was observed in a sodium cholate extract of homogenized specimens of *D. triformis* (Fig. 3-C). Quantities of *D. dipsaci* were insufficient to test for this enzyme or other cytochromes described below. The oxidation of ferrocytochrome c gave a linear relationship with 0.01 ml of extract, and a slight curve with 0.03 ml of extract. The rate of ferrocytochrome c oxidation was about 3 times as great with 0.03 ml of extract as with 0.01 ml. There was no chemical oxidation of the ferrocytochrome c in the absence of extract, and extract boiled for 10 minutes was unable to oxidize the pigment.

Absorption spectra were made on digitonin and sodium cholate extracts of *D. triformis*. Considerable difficulty was experienced as extracts became turbid following the addition of excess sodium hydrosulfite, even though pH was 8.0 or above. This turbidity also increased with time, making the readings meaningless. Digitonin extracts became more turbid than cholate extracts. The absorption spectrum illustrated in Fig. 3-D was obtained from a sodium cholate extract to which only a mg or 2 of sodium hydrosulfite had been added. Although a faint turbidity appeared on addition of the sodium hydrosulfite, periodic examination of the cuvette during the readings revealed that the turbidity did not increase with time. The absorption spectrum has a fairly sharp peak for the Soret bands around 426-430 mμ, and a much broader, less distinct peak around 550 mμ. The Soret bands were consistently distinct in all the extracts, and the band at 550 mμ was always broad. From this curve it may be seen that cytochromes b and c were present in the extracts. No peak was seen for cytochrome oxidase (cytochrome $a + a_3$) at 605 mμ.

Spectroscopic examination of massed, intact specimens of *D. triformis* also revealed the presence of

cytochrome pigments. A fairly strong absorption band was observed in the region of 546–554 mμ, indicating the band of cytochrome c. There seemed to be 2 fainter bands on either side of the strong band at 550 mμ, which might have corresponded with the α-band of cytochrome b and the β bands of cytochromes b and c. In addition, a faint but distinct absorption band was observed in the region of 600–610 mμ, indicating cytochrome a + a_3. Addition of sodium hydrosulfite to the massed nematodes increased the density of the absorption bands only slightly. Examination of a Baker's yeast suspension reduced with sodium hydrosulfite revealed strong absorption bands at the same wave lengths as seen for the nematodes.

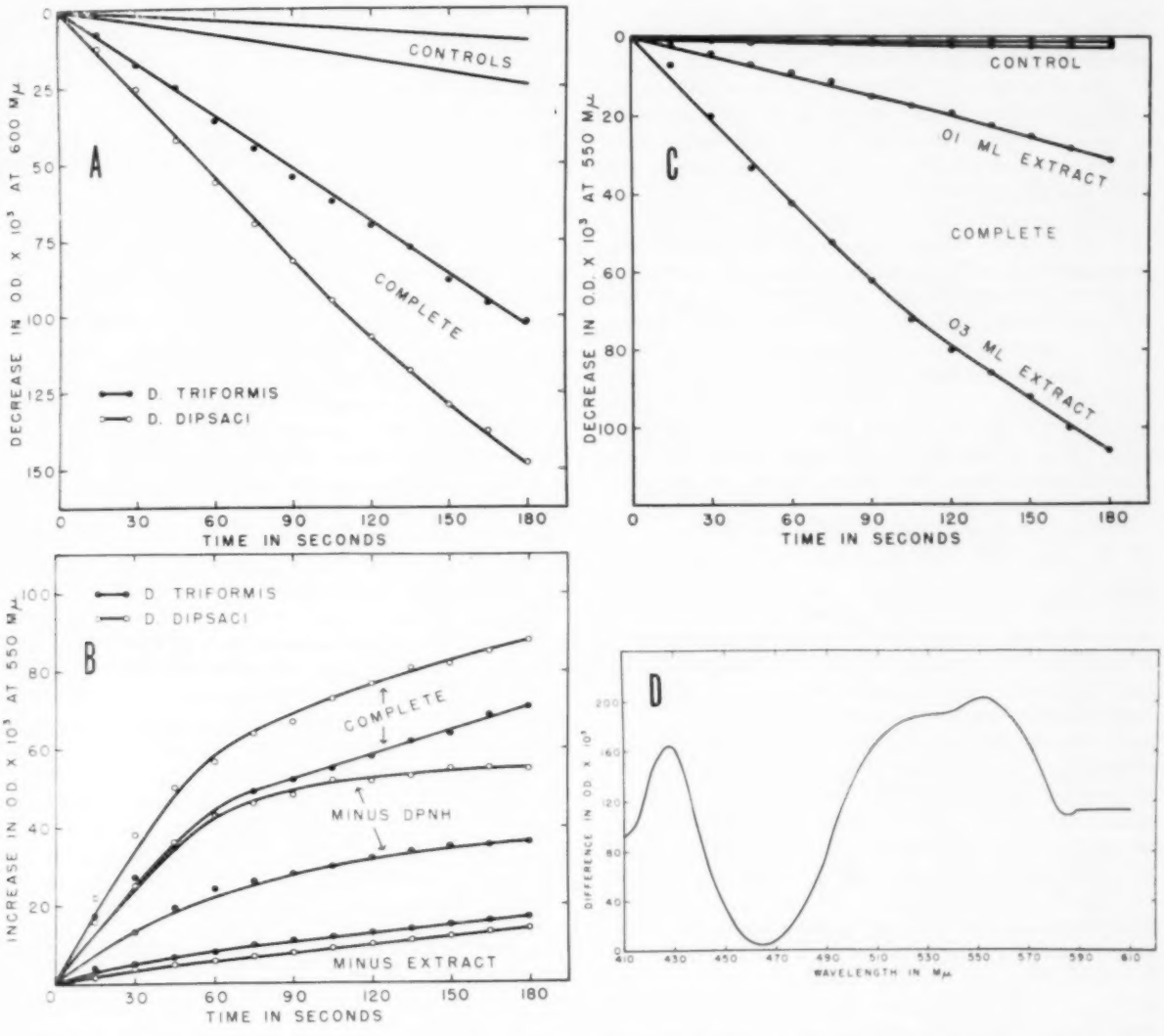


Fig. 3. Components of electron transport systems in nematode extracts. **A)** Diaphorase activity in extracts from *D. triformis* and *D. dipsaci*. The assay system (3.0 ml) contained 60 μ*M* tris-HCl buffer at pH 7.5; 0.12 μ*M* of sodium 2,6-dichlorophenolindophenol; 0.6 μ*M* DPNH; 10 μ*M* KCN; and 0.1 ml extract, dialyzed extract, or boiled extract. The complete system contained all the above components using extract or dialyzed extract; the controls were similar, but lacked DPNH or contained boiled extract. Only the curves for the limits of the control are shown. The *D. triformis* extract contained 6.6 mg protein per ml, and the *D. dipsaci* extract 5.2 mg per ml. **B)** DPNH-cytochrome c reductase activity in extracts from *D. triformis* and *D. dipsaci*. The assay medium (3.0 ml) contained 60 μ*M* glycylglycine buffer at pH 8.5; 0.1 ml aqueous 1% ferricytochrome c; 0.6 μ*M* DPNH; 10 μ*M* KCN; and 0.1 ml dialyzed extract. The complete system contained all the above components; the controls were similar, but lacked DPNH or lacked enzyme. The *D. triformis* extract contained 3.2 mg protein per ml, and the *D. dipsaci* extract 6.3 mg per ml. **C)** Cytochrome oxidase activity in a sodium cholate extract of *D. triformis*. The assay medium (3.0 ml) contained 140 μ*M* tris-HCl buffer at pH 8.5; 1.2 gamma of reduced cytochrome c; and extract or boiled extract. The complete system contained all the above components; the controls lacked extract or contained boiled extract. **D)** Absorption spectrum of a sodium cholate extract from *D. triformis* specimens. Difference in OD between reduced and oxidized extracts. A quantity of 130 was added to all the OD readings in order to make them positive.

Discussion.—Nematode homogenates and extracts were tested for the activities of 24 enzymes, 19 of which were detected. The activity levels of the different enzymes and the relative ease with which activity was demonstrated illustrate the feasibility of studying the physiology of plant-parasitic nematodes by the techniques used.

The finding of cellulolytic enzymes in *Ditylenchus* species confirms the studies made by Tracey (48). As he surmised, these enzymes probably help the nematodes penetrate plant cell walls and may explain why small nematodes, such as *Meloidogyne* larvae, are able to penetrate plant parts so easily. Demonstration of such enzymes in *Ditylenchus* spp. and the one assay with an extract of *Pratylenchus zeae* specimens suggests that Cx enzymes may be fairly common in plant-parasitic species; however, further investigations of this are needed. Maximum Cx activity may not have been obtained in these studies, since the pH of the reaction mixture averaged about 6.6 and more acid pH values have been reported as optimum for activity of these enzymes from many organisms (43).

The presence of pectinmethylesterase in these nematode species is of interest since it is reported to be quite specific for the methyl ester of polygalacturonic acid (23). This enzyme has been found in many plants and microbes, but this is the first report of its presence in nematodes.

Polygalacturonase activity was not found in any of the nematode extracts, except for possible slight activity in the one extract of *P. zeae* specimens. Tracey (48) reported slight polygalacturonase activity in one dialyzed extract of *D. dipsaci* specimens out of several extracts tested. It has been suggested (13) that plant-parasitic nematodes of *Ditylenchus* species secrete into plant tissues pectic enzymes that are responsible for the separation of cells in the infected plant part. On the basis of the studies reported here it does not appear that this enzyme is present in the nematodes, but the nematodes may interfere with plant metabolism in a way by causing the release of polygalacturonase by the plant itself, creating breakdown of cohesiveness between cells. It is also possible that polygalacturonase is present in the nematodes, but for some reason, perhaps too high a pH, was inactive in the homogenates. The optimum pH for activity of this enzyme from many organisms is lower than the pH used in these studies, which was about 6.3 (10).

Enzymes such as amylase and protease would be expected to be, and undoubtedly are, present in all plant-parasitic nematodes. Amylase activity was definitely present in extracts of specimens of both *Ditylenchus* species. The inability to demonstrate protease activity consistently in these nematodes may be partially a pH effect; an acid reaction has been reported to produce optimum hydrolysis of protein by animal-parasitic nematodes (12, 47). The pH of the 2% hemoglobin used in the present studies was 7.5, which may have been too alkaline.

Hexokinase activity seemed to be quite high in extracts of both the *Ditylenchus* species investigated. Very high hexokinase activity was found in the animal parasite *Schistosoma mansoni*, and it was suggested that 3 different hexokinases were involved (8). In the present studies it appears that *D. triformis* has at least 2 hexose kinases, and perhaps more, since glucose, fructose, galactose, and mannose were utilized in the assay used (35).

Positive lactic dehydrogenase activity was found in extracts of *D. triformis* specimens, but activity was only slight in 1 extract of *D. dipsaci*. Since *D. triformis* specimens contained this enzyme, it is probably present in *D. dipsaci* also. Because of the necessity of collecting nematodes of the latter species over a period of several days to obtain sufficient quantities for preparation of extracts and dilution of the extracts, enzyme activity may have been too low to be measured.

Activity of condensing enzyme isocitric dehydrogenase, fumarase, and malic dehydrogenase suggests that at least a partial tricarboxylic acid cycle is operative in these nematodes. Activity of α-ketoglutaric dehydrogenase was not found in extracts of *D. triformis*, and it is possible that a mechanism bypassing oxalosuccinate, α-ketoglutarate, and succinyl-Coenzyme A is operative, such as that studied in bacteria (49). However, the presence of an active isocitric dehydrogenase in extracts of specimens of both *D. triformis* and *D. dipsaci* indicates that the regular tricarboxylic acid cycle is operative. The bypass system demonstrated in bacteria combines isocitrate with acetyl-CoA, which results in the formation of succinate and malate.

Failure to demonstrate activity of several enzymes, such as alcohol dehydrogenase, glucose dehydrogenase, succinic dehydrogenase, and α-ketoglutaric dehydrogenase, in nematode extracts does not prove that these enzymes were absent. α-ketoglutaric dehydrogenase is a fairly labile enzyme, and activity might have been lost during preparation of the extracts. The assay system for succinic dehydrogenase was probably interfered with by substances present in the crude extracts that reduced the ferricyanide or decreased enzyme activity. There are in crude extracts many unknown factors that may interfere with the assays for particular enzymes.

The terminal oxidation system, at least in *D. triformis*, may proceed through the conventional cytochrome system. The presence of DPNH-cytochrome c reductase, in *D. dipsaci* extracts as well as in *D. triformis* extracts, illustrates that both nematodes have the ability to transfer electrons from DPNH to ferricytochrome c. It is interesting to compare results of extracts of *D. triformis* with those of other nematodes studied. The cytochrome system was very slightly if at all active in the terminal oxidation systems of 3 animal-parasitic nematodes (12). Cytochrome oxidase activity was present in homogenates of *Hymenolepis diminuta* Rudolphi (8). Cytochromes a, b, and c were observed spectroscopically in whole massed larvae, and cytochrome oxidase and succinate-cytochrome c reductase spectrophotometrically in larval extracts of *Trichinella spiralis* (17). Manometric

evidence of cytochrome oxidase and spectroscopic evidence of cytochrome pigments were obtained in the free-living nematode *Panagrellus silusiae* (9). Extracts of *D. triformis* contained cytochrome pigments and cytochrome oxidase and DPNH-cytochrome c reductase activities. Components of the classical cytochrome system appear absent in several nematodes, and the probable presence of this system in all nematodes cannot be assumed.

Although all the enzymes in the various pathways were not demonstrated to be present in the nematode extracts prepared, a broad picture of over-all metabolism may be seen. The Embden-Meyerhof pathway of glycolysis, the pentose monophosphate shunt, and the tricarboxylic acid cycle all appear to be present. Components of the terminal oxidation system involving the cytochromes were also found. The nematodes studied here apparently have all the respiratory systems present in other animals.

Even though plant-parasitic nematodes are considered obligate parasites, their metabolism does not seem to differ radically from that of non-parasitic organisms, on the basis of evidence thus far obtained. However, their metabolism must be different in one or more specific phases if our present concept of the specialized nature of obligate parasitism is correct. By studying nematode physiology, information may be gained that would aid in developing a medium capable of supporting their growth *in vitro*. Several free-living and animal-parasitic nematodes have been cultured on non-living media, but no plant parasites. If plant-parasitic nematodes could be propagated *in vitro*, an entirely new field would be opened for physiological studies. North Carolina State College, Raleigh, N. C.

LITERATURE CITED

1. Bernfeld, P. 1955. Amylases, α and β. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 1. Academic Press, New York, p. 149-158.
2. Bonner, W. D. 1955. Succinic dehydrogenase. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 1. Academic Press, New York, p. 722-729.
3. Bonnischen, R. K., and N. G. Brink. 1955. Liver alcohol dehydrogenase. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 1. Academic Press, New York. p. 495-500.
4. von Brand, T. 1952. Chemical physiology of endoparasitic animals. Academic Press, New York.
5. Bucher, T. 1955. Enolase from brewer's yeast. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 1. Academic Press, New York. p. 427-435.
6. Bueding, E. 1949. Metabolism of parasitic helminths. Physiol. Rev. **29:** 195-218.
7. Bueding, E., and Joan M. Mansour. 1957. The relationship between inhibition of phosphofructokinase activity and the mode of action of trivalent organic antimonials on Schistosoma mansoni. Brit. J. Pharmacol. **12:** 159-165.
8. Bueding, E., and H. Most. 1953. Helminths: metabolism, nutrition, and chemotherapy. Ann Rev. Microbiol. **7:** 295-326.
9. Cooman, E. P. 1950. Le metabolisme respiratoire d'Anguillula silusiae de Man. Ann. soc. roy. zool. Belgique **81:** 5-13.
10. Deuel, H., and E. Stutz. 1958. Pectic substances and pectic enzymes. In F. F. Nord, ed. Advances in enzymology, Vol. 20. Interscience, New York. p. 341-382.
11. Entner, N. 1957. The occurrence of the pentose phosphate pathway in Ascaris lumbricoides. Arch. Biochem. Biophys. **71:** 52-61.
12. Fairbairn, D. 1957. The biochemistry of Ascaris. Exptl. Parasitol. **6:** 491-554.
13. Filipjev, I. N., and J. H. Schuurmans-Stekhoven, Jr. 1941. A manual of agricultural helminthology. E. J. Brill, Leiden, Holland.
14. Goldberg, E. 1957. Glycolysis in Trichinella spiralis larvae. J. Parasitol. Suppl. 43, P. 44.
15. Goldberg, E. 1957. Studies on the intermediary metabolism of Trichinella spiralis. Exptl. Parasitol. **6:** 367-382.
16. Goldberg, E. 1958. The glycolytic pathway in Trichinella spiralis larvae. J. Parasitol. **44:** 363-370.
17. Goldberg, E., and L. O. Nolf. 1956. Observations on the cytochrome system in Trichinella spiralis. J. Parasitol. Suppl. 42, p. 16.
18. Herriot, R. M. 1955. Swine pepsin and pepsinogen. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in Enzymology, Vol. 2. Academic Press, New York. p. 3-7.
19. Hirschman, Hedwig. 1957. The life cycle and role of intersex in Ditylenchus triformis. (Abstr.) Phytopathology **47:** 524.
20. Horecker, B. L., and P. Z. Smyrniotis. 1955. 6-phosphogluconic dehydrogenase. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 1, Academic Press, New York. p. 323-327.
21. Kalckar, H. M. 1947. Differential spectrophotometry of purine compounds by means of specific enzymes. III. Studies of the enzymes of purine metabolism. J. Biol. Chem. **167:** 461-475.
22. Kaufman, S. 1955. -Ketoglutaric dehydrogenase system and phosphorylating enzyme from heart muscle. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 1. Academic Press, New York. p. 714-722.
23. Kertesz, Z. I. 1955. Pectic enzymes. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 1. Academic Press, New York. p. 158-166.
24. Kornberg, A. 1955. Lactic dehydrogenase of muscle. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 1. Academic Press, New York. p. 441-443.
25. Kornberg, A., and B. C. Horecker. 1955. Glucose-6-phosphate dehydrogenase. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 1. Academic Press, New York. p. 323-327.
26. Laurie, J. S. 1957. The in vitro fermentation of carbohydrates by two species of cestodes and one species of acanthocephala. Exptl. Parasitol. **6:** 245-260.
27. Levinson, H. S., and E. T. Reese. 1950. Enzymatic hydrolysis of soluble cellulose derivatives as measured by changes in viscosity. J. Gen. Physiol. **33:** 601-628.
28. Lewert, R. M., and C. Lee. 1956. Quantitative studies of the collagenase-like enzymes of cercariae of Schistosoma mansoni and the larvae of Strongyloides ratti. J. Infectious Diseases **99:** 1-14.
29. de Ley, J., and R. Vercruysse. 1955. Glucose-6-phosphate and gluconate-6-phosphate dehydrogenase in worms. Biochim. et Biophys. Acta **16:** 615-616.
30. Lowry, O. H., N. J. Rosebrough, A. L. Fair, and R. J. Randall. 1951. Protein measurements with the Folin phenol reagent. J. Biol. Chem. **193:** 265-275.
31. Mahler, H. R. 1955. DPNH cytochrome c reductase (animal). *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 2. Academic Press, New York. p. 688-693.

32. Mahler, H. R. 1955. Diaphorases. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 2. Academic Press, New York. p. 707-711.
33. Mansour, T. E., and E. Bueding. 1954. The action of antimonials on glycolytic enzymes of Schistosoma mansoni. Brit. J. Pharmacol. **9**: 459-462.
34. Massey, V. 1955. Fumarase. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 1. Academic Press, New York. p. 729-735.
35. Mehler, A. H. 1957. Introduction to enzymology. Academic Press, New York.
36. Ochoa, S. 1955. Malic dehydrogenase from pig heart. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 1. Academic Press, New York. p. 735-739.
37. Ochoa, S. 1955. "Malic" enzyme. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 1. Academic Press, New York. p. 739-753.
38. Ochoa, S. 1955. Crystalline condensing enzyme from pig heart. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 1. Academic Press, New York. p. 685-694.
39. Ochoa, S. 1955. Isocitric dehydrogenase system (TPN) from pig heart. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology. Vol. 1. Academic Press, New York. p. 699-704.
40. Racker, E. 1947. Spectrophotometric measurement of hexokinase and phosphohexokinase activity. J. Biol. Chem. **167**: 843-854.
41. Read, C. P. 1953. Contributions to cestode enzymology. II. Some anaerobic dehydrogenases in Hymenolepsis diminuta. Exptl. Parasitol. **2**: 341-347.
42. Read, C. P. 1957. The role of carbohydrates in the biology of cestodes. III. Studies on two species from dogfish. Exptl. Parasitol. **6**: 288-293.
43. Siu, R. G. H. 1951. Microbial decomposition of cellulose. Reinhold Publishing Corp., New York.
44. Smith, L. 1955. Cytochromes a, a1, a2, and a3. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology, Vol. 2. Academic Press, New York. p. 732-740.
45. Smith, W. K. 1958. A survey of the production of pectic enzymes by plant pathogenic and other bacteria. J. Gen. Microbiol. **18**: 33-41.
46. Stecker, H. J. 1955. Glucose dehydrogenase from liver. *In* S. P. Colowick and N. O. Kaplan, ed. Methods in enzymology. Vol. 1. Academic Press, New York. p. 335-339.
47. Thorson, R. E. 1956. Proteolytic activity in extracts of the esophagus of adult Ancylostoma caninum and the effect of immune serum on this activity. J. Parasitol. **42**: 21-25.
48. Tracey, M. V. 1958. Cellulase and chitinase in plant nematodes. Nematologica **3**: 179-183.
49. Wong, D. T. O., and S. J. Ajl. 1957. Significance of the malate synthetase reaction in bacteria. Science **126**: 1013-1014.
50. Zinovjev, V. G. 1957. Enzymatic activity of the nematodes parasitizing plants. (In Russian.) Zool. Zhur. **36**: 617-620. English summary.

EFFECT OF GROWING CROPS AND CROP RESIDUES ON SOIL FUNGI AND SEEDLING BLIGHTS

Lansing E. Williams and A. F. Schmitthenner

Accepted for publication June 15, 1959.
and Plant Pathology, Ohio Agricultural Experiment Station, Wooster.

Assistant Professors, respectively Departments of Botany
Published with the approval of the Director of the Ohio Agricultural Experiment Station as Journal Article No. 40-59.

SUMMARY

In a study of the effects of corn, wheat, oats, soybeans, alfalfa, and their residues on soil fungi, it was found that certain fungi were affected differently by these crops regardless of whether the growing plants or their residues were used as treatments. Other fungi were not significantly affected by the different crops but were affected differently by growing plants than by their residues. Wheat seedling blight (primarily caused by *Gibberella roseum* f. *cerealis*) was decreased most by residue treatments, and the effect of a crop was dependent upon whether the growing plant or residue was used. Alfalfa seedling blight (primarily caused by *Pythium ultimum* Trow.) was significantly affected by different crops regardless of whether the growing plant or residue was used.

Introduction.—Until recently, studies in the ecology of soil fungi have consisted mainly of floristic surveys in which the fungal populations of large areas have been characterized by a limited number of samples. This has given considerable information about the types of fungi indigenous to soil; but, if soil fungi are to be used for biological control, additional information is essential on their distributions in a variety of agricultural soils within a limited geographical area. Soil-borne pathogens are a part of the soil fungal population and are subject to change when other components of the population are affected. There is evidence that fungal populations can be altered by changes in available nutrients such as occur with crop rotation and accompanying plow-down of crop residues.

The effect of crops on soil fungi was studied in Ohio by Kommedahl and Brock (4), who found that there were significantly more fungi in 39-year continuously cropped oat soil than in soils similarly cropped to wheat or corn. Herr (3), in studying the same plots, found qualitative as well as quantitative differences in the soil fungi. Menon and Williams (5) also found qualitative differences in soil fungi between soils with different cropping histories.

This study was initiated to determine the changes brought about in the soil fungal population by certain field crops and their residues.

Materials and Methods.—Toledo silty clay was collected in equal quantities from corn, wheat, oat, soybean, and alfalfa plots. The separate samples were thoroughly mixed with each other and placed in 1-gal. glazed clay crocks. Three crop treatments were used:

TABLE 1.—*The effects of crop and crop component on the incidence of P. purpurogenum series*

Crop Component	Crop					
	Corn	Wheat	Oats	Soy-beans	Alfalfa	Mean
Growing crop	6.1*	15.2	2.4	1.4	0.0	5.0
Residue	5.6	2.1	3.0	3.4	0.0	2.8
Growing crop + residue	9.0	15.2	1.0	1.6	1.3	5.6
Mean	6.9	10.8	2.1	2.1	0.4	

Interaction LSD (0.05) = 3.8; (0.10) = 2.7
Component LSD (0.05) = 2.5; (0.10) = 2.1
Crop LSD (0.05) = 2.4; (0.10) + 2.0

* Mean arc sine percentages based on 10-plate totals converted using the inverse hyperbolic sine transformation.

TABLE 2.—*The effect of crop and crop component on the incidence of the genus Sphaeronema*

Crop Component	Crop				
	Corn	Wheat	Oats	Soybeans	Alfalfa
Growing crop	0.0*	3.1	2.9	0.0	4.5
Residue	6.4	1.2	0.0	2.7	1.4
Growing crop + residue	0.0	0.0	4.0	0.0	0.0

Interaction LSD (0.05) = 4.0; (0.10) = 3.3
Main effects were not significant.

* Mean arc sine percentages based on 10-plate totals converted using the inverse hyperbolic sine transformation.

the growing crop alone, the residue of the crop, and the combination of the growing crop with its residue. The crops were planted in excess and thinned to 1 corn, 3 soybean, or 6 wheat, oat, or alfalfa plants per crock. The residues, finely chopped dried mature plant stems and leaves, were mixed at the rate of 8 g per 9 lb. of soil (roughly equivalent to 2 tons per acre). Eight replicates were used in a randomized block design in the greenhouse. After 3 months all plants were removed and soil was taken from each crock for fungal analysis.

The techniques used to sample soil, prepare soil plates, and analyze the soil fungus data have been discussed by Schmitthenner and Williams (7). In brief they are as follows. The soil was sampled by taking 8 cores from each pot with a soil sampler. The cores from each pot were pulverized and thoroughly mixed, then a 250-mg subsample was suspended in 50 ml of 1 per cent carboxy-methyl cellulose (120 high-viscosity). A further 1:50 dilution was made in CMC, and 1-ml aliquots of this suspension were mixed in Petri plates with 10 ml of warm OAES medium, the ingredients of which were 20 g purified agar, 5 g glucose, 2 g yeast extract, 1 g sodium nitrate, 0.5 g magnesium sulfate, 1 g potassium phosphate (monobasic), 50 mg streptomycin sulfate, 50 mg Chloromycetin (chloramphenicol), 1 g oxbile, and 1 g sodium propionate per liter.

The plates were incubated for 5 days at 25°C and the fungal colonies on the incubated plates were marked and identified as soon as sporulation occurred. The fungi were identified to genus, except in the case of the Aspergilli and the Penicillia, which were identified to the groups or series as described by Thom and Raper (6, 8). Most of the genera and species isolated in this study have already been reported from Ohio soils by Williams and Schmitthenner (9). The data are summarized as the percentage of the fungi appearing on 10-plates converted using the inverse hyperbolic sine transformation for each genus and species group (1, 2, 7).

After the soil samples were removed for fungal analysis, 25 Seneca wheat and 50 Ranger alfalfa seeds were planted in each crock to determine the disease potential. After 3 weeks the surviving plants were carefully washed free of soil and examined for disease symptoms. The data for each assay crop were expressed as the percentage disease of the total number of each seed planted in each crock.

Experimental results.—The 3 treatments (the growing crop, the residue, and the growing crop plus its residue) are considered as different components of a cropping system and are referred to herein as "crop components." The incidence of each genus and species group of fungi was analyzed as a 2-way factorial analysis of variance so that the crop × crop component interaction could be studied. With only 2 fungus groups was the interaction significant. The interaction means for *Penicillium purpurogenum* series are found in Table 1, and those for *Sphaeronema* spp. in Table 2.

P. purpurogenum was very abundant in the presence

TABLE 3.—*Soil fungus groups that were affected by crop components*

Fungus	Crop Component			LSD 0.05	LSD 0.10
	Growing crop	Residue	Growing crop + residue		
Coniothyrium spp.	4.8*	3.7	2.0		2.0
Fusarium episphaeria	2.6	4.4	5.2		2.0
Fusarium moniliforme	0.6	1.5	0.0		1.0
Oospora spp.	0.0	0.8	1.2		0.6
Penicillium commune series	1.0	8.3	6.5	2.2	1.8
Penicillium decumbens series	9.9	8.8	7.0		1.6
Penicillium funiculosum series	12.7	14.7	14.9		1.7
Penicillium luteum series	0.5	1.9	0.3	1.3	1.0
Penicillium oxalicum series	0.3	0.8	0.0		0.6
Penicillium ramigena series	1.3	3.8	3.0	1.9	0.6
Penicillium rugulosum series	5.7	10.8	7.9	2.0	1.6
Stephanoma spp.	0.3	2.6	1.7	1.7	1.4
Stysanus spp.	3.4	3.8	1.6		1.7

* Mean arc sine percentages based on 10-plate totals converted using the inverse hyperbolic sine transformation.

TABLE 4.—*Soil fungus groups that were affected by growing crops*

Fungus	Crop					LSD 0.05	LSD 0.10
	Corn	Wheat	Oats	Soybeans	Alfalfa		
Absidia spp.	3.3[a]	2.2	0.4	0.8	0.0	2.1	1.7
Aspergillus clavatus group	2.3	6.8	0.6	1.4	2.0	2.6	2.1
Aspergillus flavipes group	11.2	7.5	0.2	7.4	12.5	3.1	2.5
Aspergillus fumigatus group	2.9	3.3	1.4	5.1	3.9	2.3	1.9
Aspergillus ochraceous group	4.3	3.1	5.4	4.2	9.7	3.0	2.5
Cephalosporium spp.	6.4	2.8	10.6	6.4	8.9	3.1	2.6
Cladosporium spp.	4.8	4.4	7.7	5.9	9.8	2.6	2.1
Myrothecium spp.	6.9	2.0	6.3	6.8	6.6	2.5	2.1
Penicillium oxalicum series	0.0	1.1	0.7	0.0	0.0		0.8
Rhizopus spp.	9.1	14.0	7.2	10.2	5.6	2.8	2.4
Trichoderma spp.	11.5	12.3	11.8	11.0	7.4		2.8

[a] Mean arc sine percentages based on 10-plate totals converted using the inverse hyperbolic sine transformation.

of growing wheat plants, but very infrequent in the wheat residue treatment. This fungus was also relatively abundant in all corn treatments. Corn residue increased the incidence of *Sphaeronema* most, whereas the growing corn plant had an unfavorable effect.

The fungi affected by the crop component (regardless of crop used) are listed in Table 3. The fungi affected by the crop (regardless of which component was used) are listed in Table 4. Of the 13 fungi affected by crop component, the majority were most abundant after the residue or crop + residue treatment. The Penicillia were predominant in this respect. Of the 11 fungi affected differently by the different crops, *Aspergillus* was the predominant genus. It is evident that the different crops affected the fungi listed in Table 4 differently, regardless of whether the growing crop or its residue was used.

Many other fungi were isolated and identified in this study, but were not significantly affected by any of the treatments. *Fusarium* spp., *F. oxysporum*, *F. roseum*, *F. solani*, *Nigrospora* spp., *Penicillium* spp., *P. frequentans* series, *P. janthinellum* series, unidentified Phycomycetes, *Verticillium* spp., and yeasts were abundantly isolated; *Alternaria* spp., *Arthrobotrys* spp., *Aspergillus* spp., *A. candidus* group, *A. niger* group, *A. versicolor* group, *A. wentii* group, *Botrytis* spp., *Chaetomium* spp., *F. equiseti*, *F. lateritium*, *Gliocladium roseum*, *P. canescens* series, *P. duclauxia* series, *P. lilacinum* series, *Phoma* spp., *Phycomyces* spp., and *Stachybotrys* spp. were commonly isolated; and *Actinomucor* spp., *A. flavus* group, *A. glaucus* group, *A. nidulans* group, *A. ustus* group, *Beauveria* spp., *Chaetomella* spp., *Circinella* spp., *Colletotrichum* spp., *Coniosporium* spp., *Curvulvaria* spp., *F. ciliatum*, *F. nivale*, *Fusidium* spp., *Geotrichum* spp., *Gliomastix* spp., *Gonytrichium* spp., *Haplochlara* spp., *Haplosporangium* spp., *Helminthosporium* spp., *Humicola* spp., *Isaria* spp., *Leptographium* spp., "M series" (unidentified group of moniliaceous fungi), *Monilia* spp., "P II series" (nonsporulating pink form), *P. albicans* series, *P. herquei* series, *P. lividum* series, *P. nigricans* series, *P. oxalicum* series, *Periconia* spp., *Phialophora* spp., *Pullularia* spp., *Pyrenochaeta* spp., *Sarcinella* spp., *Scopulariopsis* spp., *Sporotrichum* spp., *Stemphylium* spp., *Stigmella* spp., *Thielaviopsis* spp., and *Trichurus* spp. were occasionally isolated.

The effect of the different crops and their components on certain soil-borne pathogens were assayed by means of the seedling blight tests. The data for wheat seedling blight, primarily caused by *Gibberella roseum* f. *cerealis* (according to the Snyder and Hansen classification), are summarized in Table 5. There was least wheat seedling blight following the soybean residue treatment. The next most effective treatments were oat or wheat residue and the oat crop plus its residue. Most seedling blight occurred after wheat, alfalfa, or oat crops without their residues. The one crop + residue treatment (typical of field cropping procedure) that was most effective in reducing wheat seedling blight was oats.

The effect of the crop on alfalfa seedling blight (primarily caused by *Pythium ultimum* Trow.) was the same regardless of the component used. In the case of the corn treatments there was 47.4 per cent disease, wheat 53.3, oat 54.1, soybeans 44.7, and alfalfa 64.6. There were no significant interactions and the treatment LSD (0.05) was 7.6.

Discussion.—Undoubtedly, the major factor affecting soil-borne fungi is the amount and type of nutrients available. The fungi are dependent on organic nutrients that are almost wholly supplied by

TABLE 5.—*Percentage of wheat seedling blight following various crop and residue treatments*

Crop Component	Crop					Mean
	Corn	Wheat	Oats	Soybeans	Alfalfa	
Growing crop	62.1	75.0	68.8	62.5	71.9	68.0
Residue	59.0	50.9	53.6	40.7	64.1	53.6
Growing crop + residue	65.4	68.9	49.3	57.1	66.7	61.5
Mean	62.1	64.9	57.2	53.4	67.5	

Component LSD (0.05) = 4.3; (0.10) = 3.5
Interaction LSD (0.05) = 8.6; (0.10) = 6.7
Crop LSD (0.05) = 4.8; (0.10) = 4.0

autotrophic higher plants growing in the soil. These nutrients consist of root exudates, sloughed-off portions of growing roots, or plant residues consisting of decomposing roots or aboveground portions of plants turned under. It is of vital importance to know if one portion of the crop is more important than another in the ecology of soil-borne pathogens and saprophytes.

In a preliminary experiment Menon and Williams (5) found differences in fungi in soils cropped to corn and oats or soils treated with their residues. They found greater differences between the growing plant treatments than between the residue treatments. In this study, the effect of crops and their residues as well as the interactions of crop and residue were determined.

With 4 series of the genus *Penicillium* there was a significant effect of crop residue regardless of the crop source. This same trend was observed with other genera. In the case of other fungi, particularly the *Aspergillus* groups, crops exerted significant and different effects, regardless of the component tested.

With the Pencillia, where the residue alone gave the greatest differences, it might be hypothesized that the change is brought about predominantly by the quantity rather than the nature of organic matter. With the Aspergilli, on the other hand, where the effect of the residue was the same as that of the growing crops but where there were differences between crops, the nature of the organic matter is probably of primary importance. With the group of fungi in Table 1, the residue treatment resulted in the most differences, and with the group in Table 2, the residue was as effective as the crop or combination of crop plus residue. Therefore, it is concluded that the crop residue is the most significant aspect of a cropping system.

In general, the crop residue treatments were more effective in reducing wheat seedling blight than the crop or crop + crop residue treatments; also, oat or soybean crops were the best to precede wheat, provided their residues were plowed down. Soybeans also resulted in less seedling blight of alfalfa.

Crop treatment effects on wheat seedling blight were more similar to the crop + residue treatments in the case of wheat and soybeans, and residue treatments were more similar to the crop + residue treatments in the case of oats and alfalfa. Thus, crop has the greatest effect with the former, and residue the greatest effect with the latter. There were no differences between the 3 corn treatments.

Wheat seedling blight was best correlated with the fungi listed in Table 3, which contains several Penicillia known to be good antagonists. On the other hand, alfalfa seedling blight, which was affected by the crops rather than the components, appears to be correlated with the fungi in Table 4, but all known antagonists, with the exception of Trichoderma species, were abundant in alfalfa soil where alfalfa seedling blight was highest (L. E. Williams, unpublished data).

It was apparent from this as well as preliminary field studies (10) that the effect of a crop on the incidence of soil-borne pathogens and associated fungi in a subsequent crop is complex and dependent on the management of residues. It might be concluded that no one crop or component would be likely to build up all desirable soil fungi and decrease all soil-borne pathogens.—Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, Wooster, Ohio.

LITERATURE CITED

1. Beall, G. 1942. The transformation of data from entomological field experiments so that the analysis of variance becomes applicable. Biometrika **32:** 243-262.
2. Beall, G., and R. R. Rescia. 1953. A generalization of Neyman's contagious distributions. Biometrics **9:** 354-386.
3. Herr, L. J. 1957. Soil mycoflora associated with continuous cropping of corn, oats, and wheat. Ohio J. Sci. **57:** 204-210.
4. Kommedahl, T., and T. D. Brock. 1954. Studies on the relationship of soil mycoflora to disease incidence. Phytopathology **44:** 57-61.
5. Menon, S. K., and L. E. Williams. 1957. Effect of crop, crop residues, temperature, and moisture on soil fungi. Phytopathology **47:** 559-564.
6. Raper, K. B., and C. Thom. 1949. Manual of the Penicillia. The Williams and Wilkins Co., Baltimore, Md. 875 p.
7. Schmitthenner, A. F., and L. E. Williams. 1958. Methods for analysis of soil-borne pathogens and associated soil fungi. Ohio Agr. Expt. Sta. Botany and Plant Pathology Dept. Mimeo 29, 19 p.
8. Thom, C., and K. B. Raper. 1945. Manual of the Aspergilli. The Williams and Wilkins Co., Baltimore, Md. 373 p.
9. Williams, L. E., and A. F. Schmitthenner. 1956. Genera of fungi in Ohio soils. Ohio Agr. Expt. Sta. Research Circ. 39, 7 p.
10. Williams, L. E., and A. F. Schmitthenner. 1958. Effect of cultural practices on soil-borne pathogens of field crops and associated soil microorganisms. Ohio Agr. Expt. Sta. Botany and Plant Pathology Dept. Mimeo. 30, 42 p.

INFLUENCE OF BORON NUTRITION OF NICOTIANA TABACUM ON THE MULTIPLICATION OF TOBACCO MOSAIC VIRUS

Robert J. Shepherd and Glenn S. Pound

Accepted for publication June 19, 1959.
Supported in part by the Graduate School of the University of Wisconsin with funds supplied by the Wisconsin Alumni Research Foundation.

SUMMARY

Tobacco plants grown in liquid nutrient solutions low in boron showed characteristic deficiency symptoms and ceased growth at various times after transplanting, depending on the level of boron supplied. Virus assays with expressed saps or extracts from dried tissue samples showed lower TMV concentrations in deficient plants for 1–2 weeks following inoculation. After longer periods these differences disappeared. The virus increased in the inoculated leaves of deficient plants at a rate equal to that of nondeficient plants, and after a time exceeded the concentration in the latter.

The influence of mineral nutrition of tobacco on the multiplication of the tobacco mosaic virus (TMV) has been studied in some detail. Tobacco grown with increasingly greater concentrations of the major elements—nitrogen, phosphorus, or potassium—generally show TMV concentrations increasing in direct parallel with the effect of the nutrient on host growth (11). A similar pattern of virus multiplication occurs with certain minor-element nutrients. Helms and Pound (4) found that TMV multiplication varied directly with host growth in tobacco grown with deficiencies or excesses of zinc. With the microelement manganese, however, this relationship between mineral nutrition, host growth, and TMV multiplication does not hold; virus concentrations were highest in the most severely deficient plants (12). The same investigators (7) also studied the effect of iron nutrition of tobacco in relation to TMV multiplication. In this case, decreased virus concentrations were found only with severe deficiencies, but even in these plants the TMV concentration was not markedly less than in nondeficient plants. The present investigation extends this series of studies to include the effect of boron nutrition of tobacco on the multiplication of TMV.

Materials and methods.—Seeds of *Nicotiana tabacum* L. var. Havana 38 were germinated with Hoagland's solution in small glass-covered crocks containing coarse silica sand. Uniform seedlings were transplanted into stainless-steel trays containing sand and nutrient solution and allowed to grow to a 4- or 5-leaf stage. These plants were gently washed from the sand in order to remove the root system undamaged, again selected for uniformity, and each entire seedling rinsed well in demineralized water. These were planted directly into blackened 3½-L glass containers containing the nutrient solutions under study.

Steam-distilled water, passed through a Barnstead demineralizer, was used throughout the experiments. All containers used to culture plants and to purify and store the nutrient compounds were of soft glass. These were cleaned with a strong detergent solution followed by rinses with tap water, 5*N* HCl, and several of demineralized water. The major nutrient elements were supplied by Hoagland's 4-salt solution of $Ca(NO_3)_2$, KNO_3, $MgSO_4$, and KH_2PO_4 (6). The latter 3 compounds were purified before use by recrystallizing analytical grade salts twice from acidic solution, using redistilled ethanol in a manner similar to that described by Hewitt (5). A stock solution of $Ca(NO_3)_2$ was prepared by reacting C.P. grade HNO_3 with $CaCO_3$. Micronutrients, except iron and boron, were supplied by the A_5 solution described by Arnon (1), with the H_3BO_4 omitted. Iron was added as iron tartrate at the rate of 1 ml of a 0.5% solution per liter. Concentrations of boron ranging from 0.05 to 10 ppm were added in the form of H_3BO_4.

Test plants were inoculated with TMV when incipient boron deficiency symptoms appeared, usually 5–7 days after transplanting. Two or 3 of the lower leaves of each plant were rubbed with a dilute water solution of purified virus containing a small amount of carborundum. Plants were harvested at weekly intervals following inoculation. In the whole-plant assays, all plants of a given treatment were cut just above the surface of the nutrient container, weighed individually, and frozen in a plastic bag until used for virus assays. In some cases, plant parts were collected and assayed separately. Samples used in making virus assays on a tissue dry-weight basis were taken by removing discs of the interveinal leaf tissue with a sharp cork borer. Two to 4 discs were taken from each leaf, depending on its size, of all plants of each treatment. These samples were weighed in small tared containers, dried over $CaCl_2$ in a desiccator placed in a 50°C oven for one week, weighed again, and assayed for virus.

The virus concentration of tissue samples was determined by infectivity tests with expressed saps and ultraviolet absorbencies of purified virus preparations. The saps of frozen whole plants or tissue samples were expressed by passage of the thawed tissue through a mechanical juice extractor or by homogenizing with a mortar and pestle. The infectivities of expressed saps were compared in half-leaf inoculations of tobacco, Havana 425, using an incomplete block design. Virus for the absorbency assays was purified by two cycles of differential centrifugation after heat clarification of diluted aliquots of the expressed saps (8). The final virus preparations were resuspended in water, and aliquots diluted to a point suitable for reading on a Beckman DU spectrophotometer at 260 mμ. The results were expressed as comparable optical densities, at dilutions one-tenth that of expressed sap, or converted to weight of virus, using the slope of the curve of Takahashi (10).

The virus was extracted from dry tissue samples soaked in water for several hours. The samples were

then homogenized in small centrifuge tubes with a motor-driven polystyrene homogenizer, and the extracts clarified by slow-speed centrifugation. Following this, the cellular debris was again homogenized with water to extract whatever virus remained. These extracts were pooled, and the virus was purified and measured as with the expressed saps.

Results.—*Host response to boron.*—Tobacco plants developed typical boron deficiency symptoms soon after being transplanted into nutrient solutions low in boron. Plants grown with 0.0 and 0.005 ppm boron respectively developed deficiency symptoms about 5 and 12–14 days after being transplanted. The plants ceased growth abruptly when deficiency symptoms appeared. The first sign of deficiency was on the terminal buds. The young leaves of the terminal growing points became chlorotic and soon began to break down. These leaves and the terminal bud soon died. After a time the lateral buds broke dormancy, and grew for a short time before they too became necrotic. The partially expanded leaves immediately below the growing point became distorted, with the midribs and lateral veins showing a downward curvature (Fig. 1-B). These leaves later became thick and brittle. The vascular tissue of the upper shoot and attached leaves of deficient plants became necrotic and discolored.

Plants grown with 10 ppm boron showed only minor toxicity symptoms. A transient yellowing appeared on the smaller leaves at the top of the plants about 5–7 days after transplanting. Later, necrotic spots and margins developed on the lower leaves (Fig. 1-B).

Host response to boron nutrition was similar with both TMV-infected and healthy plants, although the

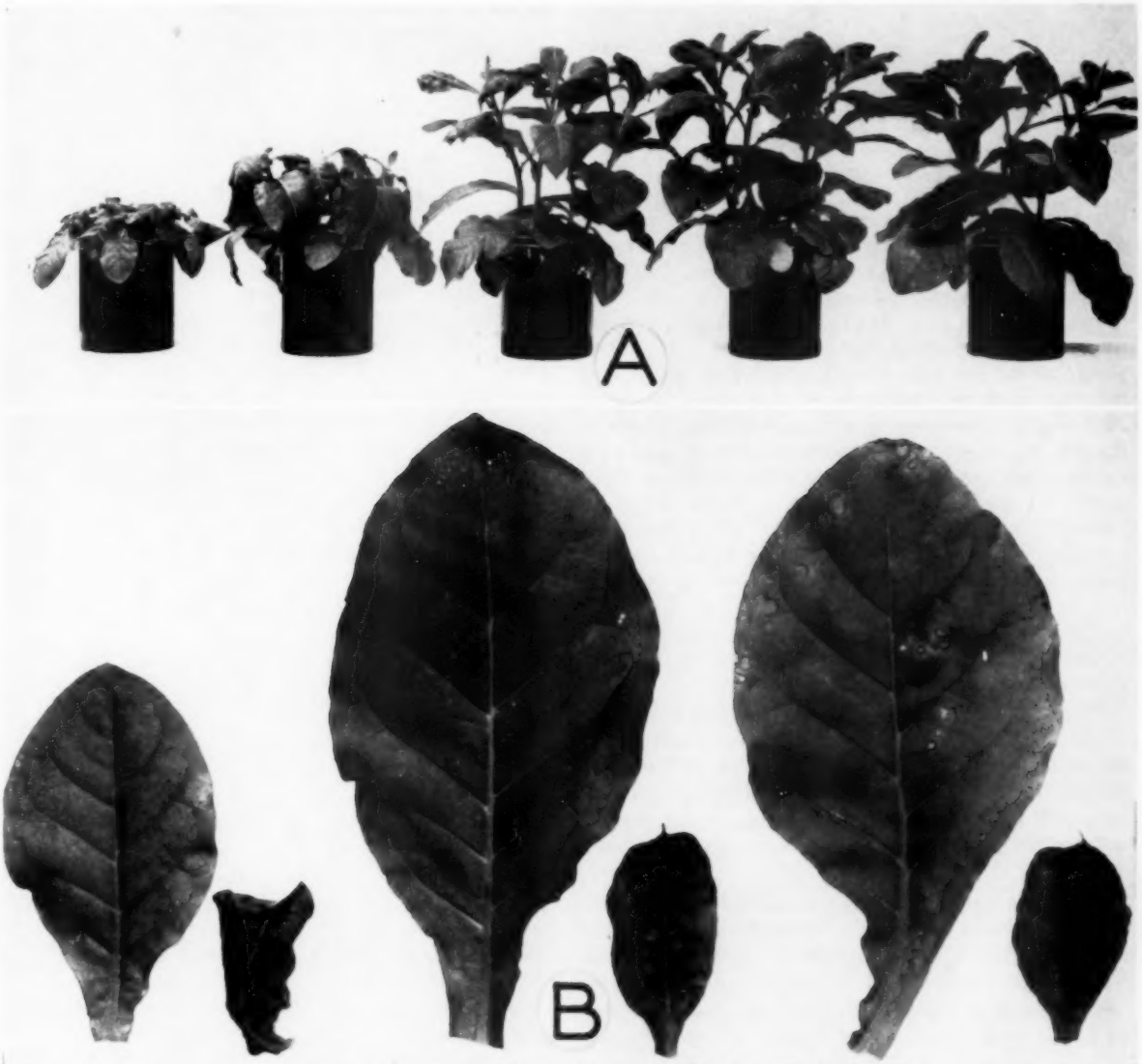


Fig. 1. Influence of boron nutrition on inoculated tobacco plants. **A)** Growth after 4 weeks in water culture containing (left to right) 0.0, 0.005, 0.05, 0.5, and 10 ppm boron. **B)** Deficiency and toxicity symptoms on inoculated leaves and tip leaves of plants grown with 0.0 (left), 0.05 (center), and 10 ppm boron (right).

TABLE 1.—*Influence of boron nutrition of Nicotiana tabacum on TMV multiplication and host growth*

Days after inoculation	Boron concentration (ppm)	Av. wet weight[a] whole plants (g)	Virus concentration: Local lesions[b]	Virus concentration: Optical density
7	0.0	6	10	0.080
	0.005	17	53	0.095
	0.05	21	48	0.105
	0.50	22	30	0.098
	10.0	19	37	0.102
14	0.0	8	74	0.241
	0.005	24	148	0.391
	0.05	32	151	0.330
	0.50	37	99	0.300
	10.0	33	161	0.395
21	0.0	10	89	0.463
	0.005	29	128	0.578
	0.05	50	116	0.377
	0.5	61	117	0.552
	10.0	47	109	0.385

[a] Average of 5 infected plants grown at each boron concentration.

[b] Average number per half leaf from 12 half-leaves in an incomplete block.

latter grew somewhat more rapidly. Boron deficiencies affect the plant portions that are growing rapidly, and growth ceased almost entirely when deficiency symptoms appeared. Thus, deficient plants were severely stunted (Fig. 1-A). Two weeks after being transplanted, or about one week after inoculation, plants at the 0.0 ppm level weighed only about one-third as much as plants grown with adequate boron (Table 1). Three weeks after inoculation, plants at the 0.0 ppm level weighed only one-fifth or one-sixth as much as nondeficient plants. Plants at the 0.005 ppm level grew at a normal rate until the deficiency appeared, and growth ceased about 10–12 days after inoculation. The slightly increased weights of deficient plants in the later harvests (Table 1) were probably due mostly to increase in the thickness of the leaves, for the average fresh weight per uniform leaf disc of deficient plants was considerably greater than that of nondeficient plants.

Plants at the 0.05 ppm level showed deficiency symptoms in only one of 5 different experiments. Generally, the growth of plants at the 0.05 ppm or 10 ppm levels was not significantly different from that of plants at the 0.5 ppm level, although the average weights at the optimum level were always greater (Table 1).

Virus concentration.—TMV increased in concentration more slowly in severely deficient plants, but after a time reached a concentration as great as in plants with adequate boron, and in some cases greater.

Five different experiments were made, with 5 levels of boron (0.0, 0.005, 0.05, 0.5, and 10 ppm) used in each experiment. Absorbency and infectivity assays with expressed saps of whole plants showed less virus in plants of the 0.0 ppm level for the first and usually the second week after inoculation (Table 1). Frequently by the second, and in almost all cases by the third week following inoculation, the virus concentration in severely deficient plants equaled that in nondeficient plants. The rapidity with which TMV increased in the plants was related to the growing temperature. During summer experiments, when daytime temperatures somewhat exceeded those of winter (about 24°C), the virus concentration usually lagged in severely deficient plants for only the first week following inoculation. Plants at the 0.005 ppm level, although developing severe deficiencies in all the experiments, did not generally show TMV concentrations significantly different from those of nondeficient plants at any time after inoculation. Plants grown with 0.05 and 10 ppm boron seldom showed virus concentrations significantly lower than those of plants at the optimum level of 0.5 ppm.

During several of the experiments, representative samples of whole plants were collected and used for virus assays on a tissue dry-weight basis. These assays confirmed the results with extracted saps (Table 2). Except for a slight lag in the plants at 0.0 ppm the first week following inoculation, the virus concentrations differed little at the various levels of boron.

Tobacco shows boron deficiency symptoms only on rapidly growing tissues, such as the growing point, the partially developed leaves, the root system, and the tissue in the region of the cambium. Thus, only a small part of the plant may be actually deficient in boron. In the saps or extracts of whole plants, therefore, the deficient tissue may be represented by only a small part of the total homogenate, and little differences may be expected in the virus content of deficient and nondeficient plants. Accordingly, various parts of deficient and nondeficient plants were collected separately and used for virus assays.

The lowermost inoculated leaves and the tip leaves of plants at the 0.0 and 0.5 ppm levels, and in some cases similar parts of plants of the 0.005 ppm level,

TABLE 2.—*TMV concentration in dry leaf disc samples taken from Nicotiana tabacum plants grown at various levels of boron*

Days after inoculation	Boron concentration (ppm)	Per cent dry weight	Mg virus per mg dry tissue
7	0.0	12.1	0.014
	0.005	10.9	0.023
	0.05	10.0	0.023
	0.5	11.5	0.019
	10.0	11.7	0.018
14	0.0	12.1	0.044
	0.005	10.1	0.038
	0.05	11.0	0.042
	0.5	11.0	0.037
	10.0	12.3	0.046
21	0.0	12.6	0.054
	0.005	10.9	0.046
	0.05	11.9	0.053
	0.5	9.7	0.059
	10.0	12.2	0.046

TABLE 3.—*Virus concentration at various leaf positions of boron-deficient and nondeficient Nicotiana tabacum plants*

Boron level and time after inoculation	Mg virus per ml expressed sap	
	Inoculated leaves	Tip leaves[a]
7 days		
0.0 ppm	2.4	2.5
0.5 ppm	2.4	4.1
14 days		
0.0 ppm	3.1	1.5
0.5 ppm	1.5	3.8
21 days		
0.0 ppm	7.9	6.3
0.5 ppm	3.9	2.2
21 days		
0.005 ppm	4.6	2.6
0.5 ppm	3.9	1.0

[a] These represent deficient tissues in the case of the plants at 0.0 and 0.005 ppm.

were collected after they had developed severe deficiency symptoms, and their virus contents were compared at intervals after inoculation. The results (Table 3) show that tissue with deficiency symptoms, represented by the tip leaves of the plants at 0.0 and 0.005 ppm, contained less virus for 1–2 weeks following inoculation, but after a time showed virus yields as great as in similar nondeficient tissues, or greater. The inoculated leaves of the plants at 0.0 ppm that were well developed before deficiency symptoms appeared, remained almost unchanged for long periods and never showed signs of impairment. These leaves showed rapidly increasing concentrations of virus following inoculation, and after a time contained a greater concentration than the inoculated leaves of nondeficient plants.

Discussion.—The role of boron in plant metabolism is not known with certainty. Experimental evidence indicates that the element is active in sugar translocation (3, 9). Sugar borate complexes are believed to form in the plant and to be able to pass through membranes more readily than nonborated sugars. In addition, it has been suggested that boron may be a constituent of the cell membranes and function in a similar manner there, by forming a temporary union with sugar molecules during their passage through the membrane.

Large amounts of sugars and other carbohydrates are known to accumulate in the leaf tissues of boron-deficient plants. The deficiency symptoms appear on the parts of the plant that are growing rapidly, such as the terminal growing point and the tissues in the region of the cambium, a feature characteristic of elements that are immobile in the plant. These are tissues with high rates of respiration and requiring large amounts of translocated carbohydrates. If boron were a constituent of membranes it would be expected to be immobile in the plant, and, once fixed in a tissue, would not again be available for transport and re-use. Thus, the plant grows normally until it exhausts the available supply of boron, abruptly ceasing growth when the supply becomes limiting. This was observed in the present experiments.

Boron is probably not involved in TMV multiplication other than in the indirect manner of favoring a more vigorous host plant. In the whole-plant assays (Tables 1, 2) the virus concentration is somewhat less in severely deficient plants for a short time following inoculation. This initial lag in the virus concentration in deficient plants may be because of fewer infective centers following inoculation, or because of a slower systemic spread of the virus as the result of poorly functioning phloem tissue, or because TMV may spread in the plant in conjunction with sugars, the translocation of which is inhibited. This mechanism would be nonfunctional in deficient plants. The latter explanation seems feasible because the spread of some plant viruses in the host, e.g., beet curly top virus and others (2), is known to be correlated with translocation of the products of photosynthesis.

In the inoculated leaves of deficient plants the TMV concentration is as great the first week after inoculation as in nondeficient plants (Table 3). Assays of the inoculated leaves of deficient plants after 2 and 3 weeks showed that considerably more virus was present than in similarly inoculated leaves of nondeficient plants. Thus, it appears that TMV multiplies at a normal rate in the inoculated leaves of deficient plants and continues to accumulate there. This apparent accumulation of virus in inoculated leaves may be because of the lack of transport of virus out of the leaves or because of a seemingly slower multiplication in rapidly enlarging nondeficient leaves.—Department of Plant Pathology, University of Wisconsin, Madison.

LITERATURE CITED

1. ARNON, D. I. 1938. Microelements in culture-solution experiments with higher plants. Am. J. Botany **25**: 322-325.
2. BENNETT, C. W. 1956. Biological relations of plant viruses. Ann. Rev. Plant Physiol. **7**: 143-170.
3. GAUCH, H. G., AND W. M. DUGGAR, JR. 1953. The role of boron in the translocation of sucrose. Plant Physiol. **28**: 457-466.
4. HELMS, K., AND G. S. POUND. 1955. Zinc nutrition of Nicotiana tabacum L. in relation to multiplication of tobacco mosaic virus. Virology **1**: 408-423.
5. HEWITT, E. J. 1952. Sand and water culture methods used in the study of plant nutrition. Commonwealth Bur. Hort. Plantation Crops, Gt. Brit., Tech. Commun. No. 22. 241 p.
6. HOAGLAND, D. R., AND W. C. SNYDER. 1933. Nutrition of strawberry plant under controlled conditions; (a) effects of deficiencies of boron and certain other elements; (b) susceptibility to injury from sodium salts. Proc. Am. Soc. Hort. Sci. **30**: 288-294.
7. POUND, G. S., AND G. W. WELKIE. 1958. Iron nutrition of Nicotiana tabacum L. in relation to multiplication of tobacco mosaic virus. Virology **5**: 371-381.
8. SCHLEGEL, D. E., AND T. E. RAWLINS. 1953. A comparison of two methods of preparing tobacco mosaic virus for spectrophotometric assay. Phytopathology **43**: 89-91.
9. SISLER, E. C., W. M. DUGGAR, JR., AND H. G. GAUCH. 1956. The role of boron in the translocation of organic compounds in plants. Plant Physiol. **31**: 11-17.
10. TAKAHASHI, W. N. 1951. Ultraviolet absorption as a measure of tobacco-mosaic virus nucleoprotein.

Phytopathology **41**: 142-145.
11. Weathers, L. G., and G. S. Pound. 1954. Host nutrition in relation to multiplication of tobacco mosaic virus in tobacco. Phytopathology **44**: 74-80.
12. Welkie, G. W., and G. S. Pound. 1958. Manganese nutrition of Nicotiana tabacum L. in relation to multiplication of tobacco mosaic virus. Virology **5**: 92-109.

SIMILAR METABOLIC ALTERATIONS INDUCED IN SWEET POTATO BY POISONOUS CHEMICALS AND BY CERATOSTOMELLA FIMBRIATA

I. Uritani, M. Uritani, and H. Yamada

Accepted for publication June 17, 1959.
This is part 25 of a study of phytopathological chemistry of sweet potato with black rot.

SUMMARY

When sweet potato is injured through some poisonous agent such as mercuric chloride, biochemical alterations are almost the same as seen in sweet potato infected by *Ceratostomella fimbriata:* Respiration is accelerated, polyphenols and coumarins increase in sound tissue adjacent to the injured part, and ipomeamaron, a sesquiterpene, is produced in the injured part. Production of ipomeamaron has been considered one of the peculiar metabolic changes in sweet potato attacked by pathogens such as *Ceratostomella fimbriata.* However, since ipomeamaron can be formed in response to chemical agents, its production may be a general reaction of sweet potato tissue in response to the injurious action of invading agents. Under certain conditions of mercuric chloride poisoning, ipomeamaron was produced not only in the injured part but also in the adjacent tissue where no browning appeared. Thus the production of ipomeamaron apparently precedes browning in the injured part attacked by the pathogen.

In the sound tissue next to tissue infected by *Ceratostomella fimbriata* (E. & H. Elliot), the pathogen of black rot of sweet potato, respiration is increased (22), and the anabolic processes *in situ* are activated, e.g., there is an increase of organic phosphate and of protein synthesis (2). Polyphenolic substances are also commonly produced in plant tissues infected by pathogens, and were observed in the sound tissue of infected sweet potato, next to the infected tissue. The polyphenolic substances were isolated and identified as caffeic acid and as caffeic acid derivatives, such as chlorogenic acid, isochlorogenic acid and pseudochlorogenic acid (21). Chlorogenic acid was already isolated from sound sweet potato by Rudkin and Nelson (11), and isochlorogenic acid was first found in coffee bean by Barnes et al (5). It was also found that coumarin substances, such as umbelliferon and scopoletin, had occurred in the sound tissue next to the infected part at a time earlier than the stage of the polyphenol production (20). The occurrence of the coumarin substances has also been found in other plant tissues attacked by pathogens (7, 16). Ipomeamaron, a bitter oily substance belonging to sesquiterpene, is produced in the injured part of the sweet potato infected by *C. fimbriata*, but scarcely any is produced in adjacent sound tissue. It was isolated by Hiura (8), and the structure was determined by Kubota and Matsuura (10).

In sweet potato infected by *Thielavia basicola* and *Fusarium* spp. the same phenomena were observed as in sweet potato with black-rot. Suzuki et al also reported a respiratory increase and production of polyphenol and ipomeamaron in sweet potato attacked by *Helicobasidium mompa* (14).

To clarify the mechanism of the above-mentioned metabolic alterations in sweet potato infected by *C. fimbriata* and the physiological role of the alterations in relation to resistance, the changes of metabolism in the sweet potato injured with poisonous agents such as mercuric chloride ($HgCl_2$) were investigated and compared.

Materials and methods.—The root of sweet potato (variety Norin No. 1) was cut into slices (2–3 cm thick), on the surface of which 2 sheets of filter paper (5 cm in diameter) were placed in layers and moistened with 4 ml of the 0.1% $HgCl_2$ solution containing 5% sodium chloride. After 12 hours another 2 ml of the solution were applied; the slices were allowed to stand at 25°C. After 24 hours of the first treatment, the surface of a sweet potato slice (1–2 mm thick) was deteriorated and killed by the agent and became soft and white; the boundary part between the dead and the sound tissues turned brown. Treatment of the 0.01% $HgCl_2$ solution produced less dramatic change, but in this case also the sweet potato tissues finally died. A solution of 0.001% $HgCl_2$ has no striking effect on the sweet potato. In each case water was applied as a control, and no noticeable change occurred. To compare the effect on sweet potato of $HgCl_2$ treatment with that of the fungus infection, sweet potato slices were subjected to inoculation of the spore suspension of *C. fimbriata* and incubated at 25°C. After 48 hours the fungus mycelium grew well on the slice surface and penetrated into the inner part as deep as 0.5–1.0 mm.

Measurement of the respiratory rate.—Rate of respiration was measured by the method of Beevers (6).

After 48 hours of the $HgCl_2$ treatment or the fungus inoculation, the thin slices (0.5 mm thick, 7 mm in diameter) were prepared from sound tissue (2–3 mm thick) next to damaged tissue. Twenty slices were put into each vessel containing 2 ml of 0.03*M* phosphate buffer solution (pH 5.5) containing 2% sucrose, and the oxygen uptake was measured.

Qualitative analysis of the polyphenols.—Caffeic acid derivatives, such as chlorogenic acid, were detected in the injured sweet potato by means of the specific color reaction. After the sweet potato tissue is soaked in sodium bicarbonate solution, it is heated up to 60–70°C, exposing the upper surface to the air. If the tissue contains the phenolic substances, it becomes green (17). The exact mechanism of the phenomenon is not clear, but the following events may be considered. During the course of heating process, sweet potato tissue is rapidly damaged and the cellular organization will also be disturbed. Polyphenol oxidase, which exists in a latent state, oxidizes freely caffeic acid derivatives to the quinones. The resulting quinones oxidize the amino acids in the presence of alkali (pH 8.0–9.0) to liberate ammonia (NH_3); the resultant reduced substances from the quinones may be condensed with the other quinones or further with NH_3 to give green pigments. In principle, this process is more or less similar to the ninhydrin reaction of amino acids (13).

Qualitative test for the coumarins.—Under ultraviolet light, umbelliferon and scopoletin respectively give blue and blue-green fluorescence. Therefore, if the tissue is placed under the ultraviolet lamp, the part involving the substances gives bright blue or blue-green fluorescence. This procedure was developed in the course of the study on coumarin substances produced in sweet potato infected by the black rot fungus (20).

Analysis of ipomeamaron.—Ipomeamaron reacts with Ehrlich's reagent (2.5 g of *p*-dimethylaminobenzaldehyde in 25 ml of alcohol and 25 ml of concentrated hydrochloride solution) and shows a pink color (15), and this fact was applied to the quantitative analysis of ipomeamaron (3). When Ehrlich's reagent is applied to the tissues, the part involving ipomeamaron shows a pink color, and the qualitative test is possible.

Results.—*Respiration.*—The respiratory rates of sweet potato treated with 0.1% and 0.01% $HgCl_2$ solutions were respectively increased by about 50% and about 20%, but that of sweet potato treated with 0.001% $HgCl_2$ was almost the same as that of the control. However, the respiration increase of sweet potato from $HgCl_2$ poisoning was relatively small compared with that from fungus infection (80–100% increase); the fact that the drift of respiration shows more or less a similar trend in both cases should be emphasized (Fig. 1).

Production of polyphenols.—It was found that polyphenolic substances including chlorogenic acid were synthesized in the sound tissue adjacent to the injured 24 hours after treatment with $HgCl_2$, and after 48 hours a more pronounced increase was observed. However, the magnitude of the formation of these substances seemed to be lower than that in sweet potato infected by the pathogen. A more precise picture of this phenomenon requires chemical analysis.

Production of the coumarins.—Twenty-four hours after treatment with $HgCl_2$, the sound part just adjacent to the injured gave a brightly blue fluorescence under the ultraviolet lamp, but its intensity was weaker than in sweet potato infected by the pathogen. This fact may indicate the formation of coumarin derivatives such as umbelliferon.

Formation of ipomeamaron.—The brown boundary between the dead part and the adjacent sound part shows pink color with the Ehrlich's reagent, and color intensity was in following order: 0.1% > 0.01% > 0.001% in consecutive manner. When sweet potato was treated with 0.1% $HgCl_2$ solution 3 times (the third treatment after 24 hours of the first 2 treatments, as explained earlier), positive Ehrlich's reaction was noticed in the adjacent inner part, which is not brown and looks sound, as well as in the brown part, which apparently died. Since indole derivatives also give a positive reaction to the reagent, only the result of qualitative analysis based on the color reaction does not always indicate that the positive substance is ipomeamaron itself. Accordingly, to isolate and characterize the positive substance, the following experiment was undertaken according to the procedure of Hiura (8) and Kubota and Matsuura (10). The dried powder (1.5 kg) of sweet potato tissue treated with $HgCl_2$ that gave positive reaction to the reagent was extracted with ether; the extract was washed with 5% sodium bicarbonate solution, 5% sodium hydroxide solution, and water, respectively, and dried with dehydrated sodium sulfate (Na_2SO_4). The ether extract was evaporated to oily substance, and finally subjected to steam distillation. The resulting distillate was extracted with ether once again, and the extract was dried with Na_2SO_4, and dried up to yellow oil (yield 19.2 g). At the final step this oily material was fractionated with distillation *in vacuo*. The main

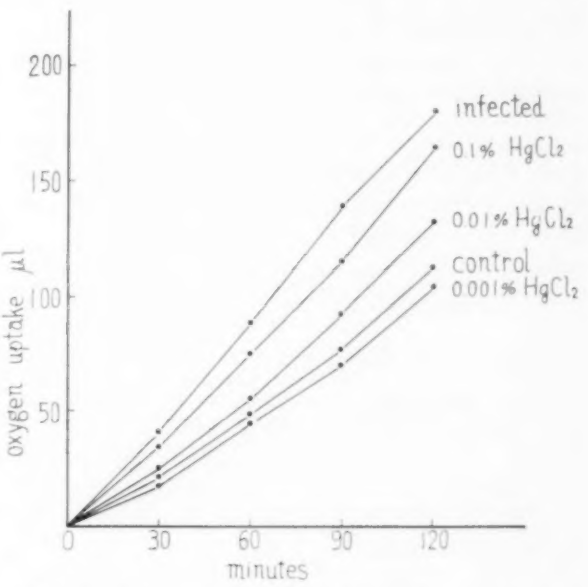


Fig. 1.—The respiration of $HgCl_2$-treated sweet potato and the infected one.

TABLE 1.—*Fractionation of the oily substances in sweet potato treated with $HgCl_2$ and infected with C. fimbriata*

Origin	Sweet potato treated with $HgCl_2$		Sweet potato infected with *C. fimbriata*	
	Boiling points (°C) 3.5 mmHg	Yields (g)	Boiling points (°C) 3.5 mmHg	Yields (g)
Distillate	100–130	3.8	100–130	0.15
	130–135	12.4[a]	130	2.0[a]
	135–153	0.6	130–167	0.21
	153–161	0.2	167–180	0.30
Residue		1.2		2.0

[a] The main fractions of the oily substances.

fraction was distilled at 130–135°/3.5 mm Hg, and the boiling point was the same as that of authentic ipomeamaron. It gave an extremely intense pink color with the Ehrlich's reagent. The results of the fractionation are shown in Table 1, together with that of sweet potato with black rot. For the final characterization, the main fraction was subjected to the next step to get semicarbazone according to Kubota's method (10). To 0.5 g of the main fraction were added 0.5 g of semicarbazide hydrochloride, 0.5 ml of pyridine, and 0.3 ml of ethyl alcohol, and the resulting mixture was allowed to stand at room temperature. After about one week, water was added to this mixture, and the resulting solid material was crystallized from dilute alcohol repeatedly. By using active carbon a pure white crystal was obtained with a melting point of 129.5–131.5°C. In parallel the authentic semicarbazone was synthesized from ipomeamaron isolated from sweet potato with black rot, and its melting point was not depressed when the both semicarbazones were mixed. The elemental analysis, calculated for $C_{16}H_{25}O_3N_3$: C, 62.5; H, 8.15; N, 13.67; found: C, 62.46; H, 7.81; N, 13.78. The main fraction and ipomeamaron were identified on paper chromatograms using three kinds of developing solvents such as methanol, butyl acetate, and butanol: acetic acid:water = 9:1:10. The Rf values were the same between two samples: 0.70 (methanol), 0.90 (butyl acetate), 1.00 (butanol:acetic acid:water = 9:1:10). In conclusion, the greater part of the substances produced in sweet potato injured with $HgCl_2$ that are positive to Ehrlich's reagent were confirmed to be ipomeamaron.

The amounts of ipomeamaron were determined twice in both the brown part of sweet potato treated three times with the 0.1% $HgCl_2$ solution and the adjacent inner part that seemed apparently sound. The amount of ipomeamaron is larger in the former part (1.35%) than in the latter (0.26%), but less than in the infected part of sweet potato with black rot (2.30%).

Treatment with the other poisonous substances.—The following chemical substances were used to investigate the responsive reaction of sweet potato in comparison with $HgCl_2$: trichloroacetic acid (TCA), a typical agent of denaturation of protein; monoiodoacetic acid (ICH_2OOH), an SH inhibitor; 2,4-dinitrophenol (DNP), a typical uncoupling agent of oxidative phosphorylation; and Na-diethyldithiocarbamate (DIECA), an inhibitor of polyphenol oxidase. The results are in Table 2. All of these poisonous substances caused polyphenol accumulation and ipomeamaron production when used in concentration high enough to induce cellular death of the part contacted with the agents, but Na-diethyldithiocarbamate did not cause polyphenol accumulation: it is assumed that the agent would also inhibit the polyphenol synthesizing system.

Discussion.—When sweet potato is infected by *C. fimbriata* and the other pathogens, in the sound part adjacent to the injured, the following events are observed: a) respiratory increase, b) polyphenol production, and c) synthesis of coumarin substances; and sesquiterpenoids like ipomeamaron are produced in the injured part. In work reported in this paper, it was confirmed that such metabolic alterations are also induced by the injury of several poisonous agents such as $HgCl_2$. It appears that the above alteration is a kind of general responding reaction of sweet potato to any injurious factors. Therefore it may not be considered that such changes of metabolism followed by the infection of *C. fimbriata* express special reactions to the particular pathogen. Ipomeamaron is formed exclusively in the infected sweet potato and not in sound sweet potato itself. So far it has not

TABLE 2.—*Ipomeamaron and polyphenol in different parts of sweet potatoes injured by poisonous chemicals*

Substances	Concentration (%)	Thickness of dead part (mm)	Ipomeamaron[a]		Polyphenol[a]
			Brown part	Sound part	
$HgCl_2$	0.1 (contg. 5% NaCl)	4.0	+++	++	+++
Trichloroacetic acid	5.0	ca 5.0	+++	++	++
Monoiodoacetic acid	0.3	6.0–7.0	+++	++	+++
2,4-dinitrophenol	0.5	3.0	+++	–	+++
Na-diethyldithiocarbamate	1.7	3.0	+	–	–

[a] Methods of the qualitative analyses are shown in materials and methods. Color reactions of ipomeamaron and polyphenol are strongest in the case of 0.1% $HgCl_2$, and the intensity is conventionally decided as +++, strong; ++, medium; +, weak. – means negative reaction.

been reported that this is formed in any other plant material. Furthermore, this substance has peculiar chemical structure and shows toxic action against *C. fimbriata* as well as sweet potato itself. For these reasons, production of ipomeamaron has been considered to be one of the most typical metabolic changes of the sweet potato induced by pathogenic infection such as *C. fimbriata* as the result of host-parasite interaction. However, from the experimental results reported here, this thought has been denied.

Ipomeamaron is produced in the infected part of sweet potato attacked by *C. fimbriata*, but scarcely any is made in the adjacent sound part. In the case of sweet potato injured with $HgCl_2$, however, under certain condition, it can be produced not only in the injured part but in the adjacent part. The fact is of value in elucidating the biochemical pathway of ipomeamaron synthesis. It is difficult to use the infected tissue as an experimental material for this purpose, because it may very rapidly lose the ability to produce ipomeamaron. On the other hand, tissue adjacent to the $HgCl_2$-injured tissue might be used since the tissue does not accompany browning and maintain the above ability.

The other biochemical characteristics in the part of sweet potato infected with black rot is the browning resulting from the oxidation of the polyphenols by polyphenol oxidase. It is very difficult to clarify the sequence of the metabolic changes in the cells suffering from penetration of the fungus, since phenomena like ipomeamaron production and browning are caused very quickly and are limited to the injured part. But, based on the fact that ipomeamaron is produced in the inner part that seems apparently sound and does not show browning in the case of sweet potato adequately treated with $HgCl_2$, it will be assumed that ipomeamaron production would have preceded browning in the infected part of sweet potato with black rot. Furthermore, the fact of impomeamaron production in sweet potato treated with toxic agents suggests that *C. fimbriata* produces the exogenous enzymes and metabolites, causing ipomeamaron production, when it attacks the tissue. The sequence of metabolic alterations in the infected part will be explained as follows. When sweet potato is attacked by *C. fimbriata*, respiration is increased in the sound part adjacent to attacked cells, and pyruvate derived from glucose-phosphate is decomposed and utilized actively through the tricarboxylic acid (TCA) cycle and fatty acid cycle. (The existence of the TCA cycle in sweet potato was confirmed by us (1).) While the pathogen penetrates into such activated cells consecutively, absorbs the nutrients *in situ*, and produces the exogenous enzymes and metabolites, the functional proteins such as mitochondria and microsoma in the host cells would be denatured, and some metabolic system concerning TCA cycle and fatty acid cycle lose the normal function and produce ipomeamaron and other essential oils as the result of the abnormal metabolism. Then, the protoplasmic function regulating the activities of enzymes such as polyphenol oxidase would be lost because of the inhibitive action of ipomeamaron (18, 19) as well as penetration of the fungus; thus, polyphenol oxidase freely oxidized the increased polyphenols such as chlorogenic acid. The resulting o-quinones react with the proteins and amino acids, thus coagulating the proteins and forming brown melanin substances. The production of ipomeamaron will be similar to the sequence of biosynthesis of rubber (4, 9) and cholesterol (12), which involves the production of mevalonic acid lactone as an intermediary substance.—Laboratory of Biochemistry, Faculty of Agriculture, Nagoya University, Anjo, Aichi, Japan.

LITERATURE CITED

1. Akazawa, T. and I. Uritani. 1954. Respiratory oxidation and oxidative phosphorylation by cytoplasmic particles of sweet potato. J. Biochem. **41**: 631-638.
2. Akazawa, T., and I. Uritani. 1955. Respiratory increase and phosphorus and nitrogen metabolism in sweet potato infected with black rot. Nature **176**: 1071-1072.
3. Akazawa, T., I. Uritani, and T. Hirai. 1957. Phytopathological study of black-rotten sweet potato. Part 24. The relation of metabolic activation to resistance in black-rotten sweet potato. J. Agr. Chem. Soc. Japan **31**: 182-185.
4. Bandurski, R. S., and H. J. Teas. 1957. Rubber biosynthesis in latex Hevea brasiliensis. Plant Physiol. **32**: 643-648.
5. Barnes, H. M., J. R. Feldman, and W. V. White. 1950. Isochlorogenic acid. Isolation from coffee and structure studies. J. Am. Chem. Soc. **72**: 4178-4182.
6. Beevers, H. 1953. 2,4-Dinitrophenol and plant respiration. Am. J. Botany **40**: 91-96.
7. Best, R. J. 1948. Studies on a fluorescent substance present in plants. Part 3. The distribution of scopoletin in tobacco plants and some hypotheses on its part in metabolism. Australian J. Exptl. Biol. Med. Sci. **26**: 223-230.
8. Hiura, M. 1943. Studies on storage and rot of sweet potato (2). Rept. Gifu Agr. Coll. **50**: 1-5.
9. Johnson, J. A., D. W. Racusen, and J. Bonner. 1954. The metabolism of isoprenoid precursors in a plant system. Proc. Natl. Acad. Sci. **40**: 1031-1037.
10. Kubota, T., and T. Matsuura. 1953. Chemical studies on the black rot disease of sweet potato. J. Chem. Soc. Japan **74**: 101-109, 197-199, 248-251, 668-670.
11. Rudkin, G. O., and J. M. Nelson. 1947. Chlorogenic acid and respiration of sweet potatoes. J. Am. Chem. Soc. **69**: 1470-1474.
12. Rundney, H. 1955. The synthesis of β, β-dimethylacrylic acid in rat liver homogenates. J. Am. Chem. Soc. **77**: 1698-1699.
13. Schonberg, A., R. Maubacher, and A. Mostofa. 1948. Degradation of α-amino acids to aldehydes and ketones by interaction with carbonyl compounds. J. Chem. Soc. 176-182.
14. Suzuki, N., K. Kasai, Y. Yamazaki, T. Araki, S. Toyoda, and T. Takanashi. 1957. Studies on the violet root rot of sweet potatoes. Bull. Nat. Inst. Agr. Sci. Japan No. **8**: 1-173.
15. Suzuki, N., and I. Uritani. 1952. On some constituents showing positive reaction by Ehrlich's aldehyde reagent in brown-rotten tissues of sweet potato. Ann. Phytopath. Soc. Japan **16**: 54-56.
16. Tamari, K. 1957. Biochemical studies on Piricularia oryzae. Shokubutsu Boeki (Plant Protection in Japanese). **11**: 233-239.
17. Uritani, I. 1953. Phytopathological chemistry of black-rotten sweet potato. Part 10. The mechanism for greening occurred on the sound part next to the

injured, when dipped in sodium bicarbonate solution (I). J. Agr. Chem. Soc. Japan **27**: 781-785.
18. URITANI, I., T. AKAZAWA, AND M. URITANI. 1954. Increase of respiratory rate in sweet potato tissue infected with black rot. Nature **174**: 1060.
19. URITANI, I., AND T. AKAZAWA. 1955. Antibiotic effect on Ceratostomella fimbriata of ipomeamaron, an abnormal metabolite in black rot of sweet potato. Science **121**: 216-217.
20. URITANI, I., AND I. HOSHIYA. 1953. Phytopathological chemistry of the black-rotten sweet potato. Part 6. Isolation of coumarin substances from sweet potato and their physiology. J. Agr. Chem. Soc. Japan **27**: 161-164.
21. URITANI, I., AND M. MIYANO. 1955. Derivatives of caffeic acid in sweet potato attacked by black rot. Nature **175**: 812.
22. URITANI, I., AND S. TAKITA. 1953. Phytopathological Chemistry of black-rotten sweet potato. Part 8. Abnormal increase of the respiration of the injured sweet potato. J. Agr. Chem. Soc. Japan **27**: 168-174.

THE ANTIGENIC CHARACTERISTICS AND THE RELATIONSHIP AMONG STRAINS OF ALFALFA MOSAIC VIRUS

J. B. Bancroft, Ellen L. Moorhead, J. Tuite, and H. P. Liu

Respectively Assistant Professor, Purdue; Plant Pathologist, U.S.D.A.; and Assistant Professor and Graduate Assistant, Purdue. Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana; and Crops Research Division, ARS, U.S.D.A., Lincoln, Nebraska.

We thank Drs. Fulton, Rozendaal, Zaumeyer, Steere, Oswald, Kreitlow, and Milbrath for kindly sending 22 of the 24 isolates listed in Table 4.

Accepted for publication June 22, 1959.

Journal Paper No. 1424 of the Purdue Agricultural Experiment Station, and published with the approval of the Director as paper No. 931, Journal Series, Nebraska Agricultural Experiment Station. Cooperative investigation, Purdue Agricultural Experiment Station, Nebraska Agricultural Experiment Station, and Crops Research Division, ARS, U. S. D. A.

SUMMARY

Alfalfa mosaic virus (AMV) was studied serologically. The antibody responses of rabbits injected intravenously with saline suspensions of virus and intramuscularly with an emulsion of virus in "incomplete" Freund adjuvant (mineral oil) were compared by complement fixation and ring-interface precipitin tests. Strains of AMV differing widely in pathogenicity and geographical origin were generally closely related serologically to the type strain used in this work. Cross-reaction tests with 8 other plant viruses indicated that AMV is a distinct serological entity. Results from cross-protection tests were always in agreement with those from serological tests. Host response studies showed that different AMV isolates may be conveniently grouped on the basis of systemic reactions on bean and cowpea.

Alfalfa mosaic virus (AMV), which is very variable (9, 23), may cause important crop losses (6, 7). In Indiana, the virus is prevalent in alfalfa and Ladino clover but not in red clover. Unfortunately, the prevalence of the virus is not always easy to estimate, because the symptoms produced are very diverse and because, as previously noted (21), symptom expression is greatly influenced by environment. For example, of 686 second-year alfalfa plants examined in May, 1957, 93% were found infected on a basis of results obtained by inoculating representative plants showing different symptom types to differential hosts. Yet, in October of the same year and in the early spring of 1958, fewer than 20% of the 686 plants showed symptoms, and these were all of the bright-yellow streak type. When plants showing different types of symptoms were indexed in the greenhouse on bean (*Phaseolus vulgaris* L. var. Bountiful), cowpea (*Vigna sinensis* (Torner) Savi var. Blackeye) and tobacco (*Nicotiana tabacum* L. var. Connecticut Havana 423), the variety of symptoms produced led us to wonder whether we were always isolating AMV. Cross-protection tests indicated that our original identifications were correct, but these tests were somewhat tedious and serological procedures seemed more suitable. Accordingly, one of our isolates (AC-106) (Catalogue and Registry of the Plant Viruses, 2nd Edition) was purified and characterized (1, 2) and used as an antigen.

The work presented here is an evaluation, first, of the antigenicity of AMV and, second, of the serology and definition of the AMV groups as represented by isolates differing in pathogenicity and geographical origin.

Materials and methods.—*Virus isolates.*—Nineteen AMV isolates, representing different symptom types, were collected from various locations in Indiana. These isolates were all inoculated to tobacco, bean, and cowpea. Twelve were checked by cross-protection tests, and all were examined serologically (Table 2). In addition, purified preparations of 22 previously identified isolates from various other locations were examined serologically. These latter isolates were in most cases identified by their donors as separate strains by the names or numbers listed in Table 4. The pathogenicity of the other strains, except yellow patch, yellow dot, and yellow mosaic strains (23), has not been described in detail in the literature.

Preparation of antigen and antiserum.—The virus used as the antigen was prepared from infected tobacco by differential ultracentrifugation, by the schedule described by Bancroft and Kaesberg (2). The weight of virus used as the antigen for the immunization studies was determined from a standard curve of dry weight (105°C for 24 hours) plotted against optical density (1 cm light path) at 260 mμ.

After a sample of normal serum was obtained from

each rabbit, 2 immunization procedures were used to obtain virus-specific serum. In one procedure, 8 rabbits received 1–3 intramuscular injections of virus emulsified in "incomplete" Freund adjuvant (17) from the Difco Laboratories, Detroit, Michigan. In the other procedure, 2 rabbits received 3–5 intravenous injections of virus suspended in a physiological saline solution. The injection schedule is described in a later section. All animals were bled periodically from the ear vein, and the serum, obtained by standard methods, was stored at −20°C. One of the animals that received the intravenous injections was bled from the heart, and the antiserum obtained from this animal was used in studies of relationships among the strains described here.

Serological test procedures.—The complement fixation technique was that devised for Rickettsia (16), which, with a few modifications, was found adequate for plant viruses (12, 13). Sheep red blood cells were obtained from the Brown Laboratories, Topeka, Kansas, or from the Animal Pathology Department, University of Nebraska. Complement and amboceptor were obtained from Merck, Sharp, and Dohme Co., Philadelphia, Pennsylvania.

A grid titration was set up with a serum sample from each rabbit taken during a period of maximum antibody response. These were in the second or fourth week from one of the rabbits injected intravenously with a saline suspension of virus, and during the fourth or sixth week from 7 of the rabbits injected intramuscularly. Nine twofold dilutions of antigen beginning at 1:64 were reacted with 5 twofold dilutions of serum beginning at 1:32 or 1:64. The greatest dilution of antigen giving a 4-plus, or complete fixation, endpoint with the most dilute serum contained the optimum ratio of antigen and antibody. This dilution of antigen contained the antigenic unit as determined by calculations based on the original weight of virus in the solution. Sera from 8 rabbits were examined in 2 groups of 4 samples for the complement fixation studies. Since the interval between the acquisition of the groups of sera was several months, 2 different antigen preparations were used. The antigenic units for these preparations were calculated to be 1.7 μg/ml and 2.6 μg/ml. A constant dilution containing 2 antigenic units was used with varying dilutions of all the serum samples from each rabbit in order to determine the amount of antibody present with respect to time after inoculation.

The amounts of antibody in the sera were also determined by ring-interface precipitin tests. The ring tests were made by making twofold dilutions of antiserum in 0.002*M* phosphate-buffered physiological saline, pH 7.0, containing 10% glycerine.[1] The antiserum was added to chemically clean small tubes made from soft glass tubing (od 6mm) and the virus, in buffered saline, was layered over this. Previous semigrid and grid titrations had shown that a virus concentration of 15 μg/ml would be suitable for all animals in erms of antigen economy and speed and clarity of reaction. After incubation periods of 1 and 2 hours at room temperature, the sides of the tubes were wiped with mineral oil[1] and readings were made in a dark room using strong back lighting. The antibody titer was recorded as the highest antiserum dilution at which a positive reaction occurred. A thin white line at or near the interface indicated a positive reaction (Fig. 1). The tubes were used only once and then discarded.

Serological relationships were tested by using the ring-interface precipitin technique. If crude sap was to be tested, the extracts obtained from tobacco were clarified by freezing and centrifugation, diluted to 1:100 or 1:200, and layered over a 1:16 dilution of antiserum. In some cases, virus was detected at antigen dilutions of 1:6400 from crude sap. This value is in excess of the infectivity dilution endpoint and can be explained by taking into account the large amount of noninfectious serologically active protein (1, 2) associated with AMV. Semiquantitative comparisons were made of purified preparations of the 24 isolates listed in Table 4. The purified preparations, scanned on a Beckman DU spectrophotometer, all showed similar absorption curves in ultraviolet light. The preparations were all adjusted to the same optical density at 260 mμ, and twofold dilutions were reacted against aliquots from the same lot of 1:16 diluted antiserum. The error resulting from equalizing the virus preparations spectrophotometrically is undoubtedly insignificant when it is considered that errors of possibly 50% are introduced when twofold dilutions are used.

Controls.—The controls normally included in standard serological procedures were always employed. It was noted that false rings formed in the ring test if weights of antigen above 0.1 mg/ml were used. These rings formed even on glycerine and were rather diffuse and not so sharp as true rings, but they

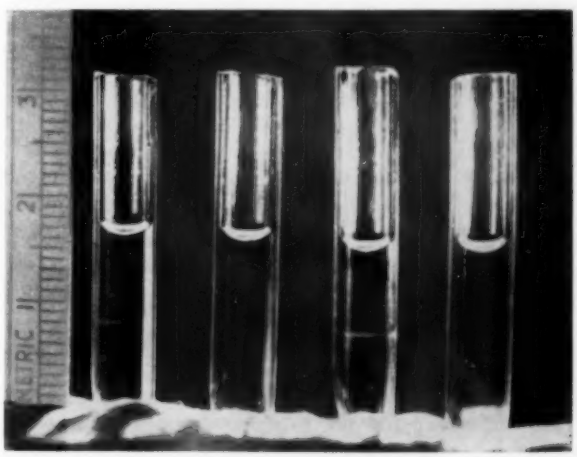


Fig. 1. The ring-interface precipitin test. A positive reaction against clarified sap from AMV-infected plants is seen in the first and third tubes, and a negative reaction against clarified sap from healthy plants is seen in the second and fourth tubes.

[1] The authors thank Dr. L. M. Black for these suggestions.

TABLE 1.—*Reciprocal of the antiserum dilution endpoint obtained with AMV and 9 other plant virus antisera when titrated with homologous and heterologous antigens as determined by complement fixation technique*

Virus antisera	AC no. or source	Titration with purified AMV antigen	Titration with purified homologous antigen
Alfalfa mosaic	106	512	
Tobacco mosaic	3	<4[a]	512
Southern bean mosaic	18	<4	1024
Tobacco ringspot	Nebraska	<4	256
Potato X	Nebraska	<4	512
Tobacco necrosis	Nebraska	<4	256
Brome mosaic	66	<4	256
Barley stripe mosaic	69	<4	128
Cucumber green-mottle mosaic (CV3)	Dr. C. A. Knight	<4	1024
Alfalfa mosaic	Nebraska	1024	512

[a] In complement fixation test, serum was anticomplementary at dilutions less than 1:4, and therefore could not be evaluated.

obscured the true rings. In addition, if unbuffered saline that had been stored at room temperature for some time was used, false rings formed very rapidly. Fresh buffered saline is advisable if no refrigeration is available. The antisera did not react against either crude or concentrated preparations from healthy tobacco when tested by the ring-test and double-diffusion agar techniques (9).

The complement fixation test was used for cross-reactions with AMV antigen and antisera specific for 9 other plant viruses. Both homologous and heterologous antiserum dilution endpoints of these sera are presented in Table 1. The data showed that no cross-reactions occurred between AMV antigen and 8 other plant virus sera. The one serum that did react with AMV antigen was prepared from a virus isolated from diseased soybean plants in Nebraska. This virus had been shown in unpublished studies[2] to belong to the AMV group. Tobacco mosaic virus, tobacco ringspot virus, and AMV gave positive reactions against homologous, but not against heterologous, sera in the ring test. The other viruses were not tested by this method.

Cross-protection.—Cross-protection tests were made on bean and cowpea. If the virus to be identified produced well-defined necrotic lesions, it was used as the challenge virus, and Indiana 61, which produces chlorotic spots and a systemic reaction, was used as the established virus. If, on the other hand, the virus to be identified did not produce well-defined necrotic lesions, it was used as the established virus and the yellow patch strain of AMV was used as the challenge virus.

Experimental results.—*Antigenicity of alfalfa mosaic virus.*—Fig. 2 compares animal responses, determined by complement fixation and ring-interface precipitin tests, to intramuscular and intravenous injections. A rabbit, injected intramuscularly, received two 10-mg doses of AMV emulsified in adjuvant 3 weeks apart followed by a 5-mg injection during the sixteenth week. The maximum antibody titer was reached by the fourth week. Thereafter, the titer dropped until the tenth week and remained constant until the 5-mg dose increased it to the maximum level. The serum from a rabbit that was injected intravenously with 5-mg doses of antigen in saline yielded a typical short-lived response, with maximum antibody titer occurring in the second week. A booster dose of antigen given 13 days after the last injection of the primary series had an effect similar to that described above. A second rabbit, injected at a later time with a virus-saline solution, showed an antibody response curve similar to that in Fig. 2.

Two other rabbits were injected intramuscularly

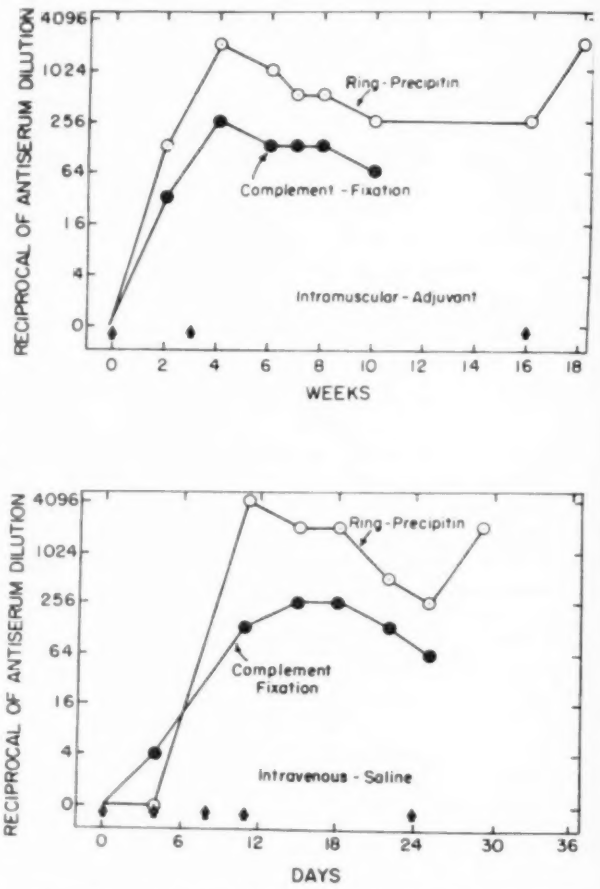


Fig. 2. A comparison of animal response to virus-adjuvant (mineral oil) intramuscular injections and virus-saline intravenous injections as measured by the complement fixation and ring-interface precipitin methods. The first 2 arrows on the upper graph along the time scale refer to 10-mg injections of AMV, whereas the last one and all the arrows on the lower graph along the time scale refer to 5-mg injections.

[2] Dr. W. B. Allington and Dr. R. W. Staples.

TABLE 2.—*Description of some alfalfa mosaic isolates from Indiana*

Indiana isolate No.	Reaction of Bean		Cowpea		Cross-protection	Serological reaction
	Local	Systemic	Local	Systemic		
3	+[a]	+[b]	x[c]	–	+	+
5	x	–	x	–	NT[d]	+
6	+	+	+	+	NT	+
7	+	+	+	+	+	+
8	x	–	x	–	+	+
39	+	+	+	+	NT	+
61	+	+	+	+	+	+
83	+	+	+	+	+	+
84	+	+	+	+	+	+
85	+	+	+	+	NT	+
86	x	–	x	–	+	+
87	+	+	+	+	NT	+
91	x	–	x	–	+	+
92	x	–	x	–	+	+
94	+	+	+	+	NT	+
104	+	+	+	+	+	+
105	+	+	x	–	NT	+
106	+	+	x	–	+	+
109	+	+	+	+	+	+

[a] The + under a local-reaction heading designates any type of reaction other than a well-defined necrotic local lesion on the inoculated primary leaves.

[b] The + under a systemic reaction heading designates any systemic reaction on the uninoculated trifoliolate leaves.

[c] The x under a local-reaction heading designates a well-defined necrotic local lesion on the inoculated primary leaves.

[d] The NT designates no test.

with single large (19–29 mg) doses of emulsified virus. Both of these animals responded similarly to the rabbit that received 2 initial injections, in that the antibody-profile curves were about the same shape, with maximum titers at the 4- to 6-week period. Another animal was injected with incompletely emulsified antigen and responded poorly in a manner suggestive of a single injection of antigen given in saline. However, this same antigen was effective in raising the antibody titer when injected into another animal that previously had received properly emulsified virus.

Two of the animals used early in this work developed large cysts after an intramuscular injection with emulsified virus. These cysts most likely resulted from impurities associated with the virus preparations. Later antigen preparations were subjected to additional steps in the purification procedure, and cysts did not develop.

The results obtained independently by complement fixation and ring-precipitin tests always showed the same type of response curve in the animals and are considered to be in good agreement. On an average, based on 38 comparisons, the titers found by complement fixation methods were about 2 dilutions less than those obtained by ring tests. Antiserum dilution endpoints from the precipitin tests were determined with 15 μg/ml of antigen, and the final readings were recorded at the highest positively reacting antiserum dilution. Complement fixation tests, on the other hand, were run with 3.5–5.2 μg/ml of virus and the final readings were based on the highest antiserum dilution that gave a complete fixation reaction.

Description of isolates from Indiana.—Symptoms on alfalfa plants from which AMV was isolated varied considerably, from greenish mosaics to faintly yellowed leaves, from bright-yellow streaks to mild vein clearing, and from leaf puckering to a general stunting of plants and shortening of the internodes. Considerable variation in symptomatology was also found on bean, cowpea, and tobacco. As can be seen in Table 2, two main types of lesions were noted on bean and cowpea: 1) a well-defined necrotic lesion on the inoculated primary leaves, relatively stable in appearance in spite of environmental change, was usually associated with an isolate that did not become systemic on the uninoculated trifoliate leaves; and 2) a less well-defined type of reaction, sensitive in appearance to environmental change, which appeared on the inoculated primary leaves and which was usually associated with systemic spread to the trifoliate leaves. These data, based on 2–9 replications at different times for each isolate, are in agreement with those reported by Milbrath and McWhorter (11) on cowpea. The spreading type of reaction had many variants, such as a diffuse necrotic type that spread along adjacent veinlets, or a chlorotic ring-spot type or merely a diffuse area of chlorosis. Only systemic strains were isolated from alfalfa plants showing striking yellow leaf markings (23) and vein clearing. Systemic isolates were not, however, limited exclusively to alfalfa exhibiting these symptoms. AMV was isolated from the tips of systemically infected bean and cowpea plants, and isolate 39 was identified serologically directly from bean tip leaves. Isolates from the tip leaves of the plants were also used to guard against mixtures. Infections from single lesions corroborated the evidence that there were no mixtures. All isolates were checked serologically from tobacco and were shown to belong to the AMV group. The 12 isolates used in cross-protection tests showed reactions consistent with the serological data. From the information gained in these tests, it appears that a convenient way to group isolates of AMV would be to note whether an isolate became systemic on either bean or cowpea or on both hosts. The systemic reaction, which sometimes requires about four weeks to appear, is easily repeatable between 70 and 80°F.

In one experiment, bean and cowpea were inoculated with 35 collections of AMV-infected alfalfa plants showing a good cross section of symptom types. In

TABLE 3.—*Prevalence in Indiana of 35 isolates of AMV and their symptom expression on bean and cowpea*

Type	Prevalence (%)	Bean	Cowpea
1	40.0	Systemic	Systemic
2	36.7	Local	Local
3	16.7	Systemic	Local
4	6.6	Local	Systemic

TABLE 4.—*Reaction of standardized[a] antigens of various geographically isolated strains of AMV with homologous antiserum specific for the Indiana type isolate (AC-106) as measured by the ring-interface precipitin test*

Strain and source	AC Number[b]	Antigen dilution endpoint (μg/ml)	Strain and source	Antigen dilution endpoint (μg/ml)
Indiana type	106	0.75	Oregon 1[c]	0.75
Indiana 39	109	0.75	Oregon 7	0.75
Beltsville isol.[d]	105	0.75	Oregon 8	0.75
Yellow Patch[e]	107	0.75	Oregon 9	0.75
Yellow Dot[e]	90	0.75	Oregon 17	0.75
Yellow Mosaic[e]	91	1.50	Oregon 20	0.75
Dutch Virulent[f]		0.75	Oregon 24	0.75
Dutch Avirulent		1.50	Oregon 29	0.75
Berkeley isol.[g]	108	0.75	Oregon 30	0.75
California 15[h]		0.75	Oregon 32	3.00
California 16		1.50	Oregon 34	0.75
Kreitlow[i]		0.75	Oregon 54	0.75

[a] Antigen preparations contained 0.1 mg/ml in original solution.

[b] Culture numbers assigned in the Catalogue and Registry of the Plant Viruses, 2nd Ed., 1959. Washington, D. C.

[c] Dr. J. A. Milbrath. None of the Oregon strains have AC numbers.

[d] Dr. R. W. Fulton.

[e] Dr. W. J. Zaumeyer. The Yellow Patch was originally from Dr. K. W. Keitlow.

[f] Dr. A. Rozendaal. Virulent and avirulent refer to symptoms produced on potato.

[g] Dr. R. L. Steere (received in pure form). Originally isolated by Dr. C. E. Yarwood.

[h] Dr. J. W. Oswald.

[i] Dr. K. W. Kreitlow.

Table 3 it can be seen that, from this kind of collection, isolates which give the same type of response, either local or systemic, on both bean and cowpea rather than on either bean or cowpea are the most prevalent.

Serological comparison of geographically isolated strains.—The plants infected with the isolates listed in Table 4 were harvested 11 days after inoculation. The sap was expressed, and preliminary serological tests indicated that all the strains reacted against our antiserum. Considerable symptom differences were noted in tobacco, but symptom severity and virus content, as estimated by measuring the final yield of pure virus, were not necessarily related. For example, a very severe strain such as alfalfa yellow mosaic virus produced only about one quarter as much virus as did AC-109, a strain that produces only mild symptoms on tobacco.

An evaluation of the reactivity of purified preparations of the various strains with the ring-test indicated that serological procedures short of cross absorption or optimum antigenic unit determinations (20) probably would be inadequate for differentiating most strains. Only 4 strains of the 24 tested showed endpoints different from the homologous reactions with AC-106 antiserum. It thus appears that there probably is considerable serological similarity among strains differing in geographical origin and pathogenicity.

Discussion.—Antibody response was good in rabbits immunized with AMV. In ability to produce antibodies, this virus could be grouped with such plant viruses as barley stripe mosaic (12), brome mosaic (12), tobacco ringspot (13), and tobacco necrosis (13). These viruses, when injected intramuscularly in an emulsion, give relatively short periods of maximum antibody production in contrast to cucumber green-mottle mosaic (CV3) (13), tobacco mosaic (13), southern bean mosaic (13), and potato X viruses (13), which give long periods of maximum response. Possible reasons for an antibody curve of the AMV type have been discussed previously (12).

Clearly, intramuscular adjuvant injections were preferable to intravenous injections since high titers lasted longer. In addition, as noted elsewhere (12), the intramuscular injections with adjuvant are much less troublesome than repeated intravenous injections.

The ring-interface precipitin test proved very useful. Its advantages over the standard tube precipitin tests are its sensitivity (2), economy, and readability. In addition, since the antigen and antibody diffuse toward each other, the effects of excess of either solution are minimized. The ring-test appears to give results consistent with those found by complement fixation methods, yet it is a less laborious procedure.

Zaitlin et al (22) reported using the ring-test with certain orchid viruses. They found, using antigen free of healthy plant constituents, that nonspecific reactions developed at periods over 20 minutes when chloroform-cleared sap was tested. This effect is not, in our experience, inherent in the method but might be explained by surmising that normal components of the orchids may have been precipitated by the clearing solvent. The ring-test has been used successfully with viruses obtained from plants and insects (3).

There is every evidence that serological groups, which should be of inferior rank to the generally larger morphological groups in plant-virus classification, are the most easily usable units in virus identification. Although some animal viruses give heterophilic reactions (5), none have been found with the plant viruses as yet, and the interpretive difficulties encountered with some of the animal virus groups (4) are not apparent at this time. Consequently, there can be little doubt, as intimated elsewhere (15, 19) and as indicated by our data, that AMV comprises a distinct and usable serological group. This conclusion is supported by cross-protection tests and by the observation that AMV particles have a unique morphology (1).

Our data show that semi-quantitative ring precipitin tests do not differentiate most strains of AMV. This result is not surprising when one considers that some strains of tobacco mosaic virus cannot be differentiated by quantitative precipitin tests (8). However, as is the case with such strains of TMV, differences among the AMV strains probably could be determined by cross-absorption tests (8) or on the basis of the optimum antigenic unit (14, 20). These tests would necessarily be laborious. Examinations of the morphology and sedimentation behavior of 2 widely

differing isolates of AMV (AC-106, AC-109) have shown no differences between them (2). For most purposes, the use of indicator plants for classifying strains or pathogenic groups is the most practical method. The criteria for forming such groups are, of course, subjective, but there is no good reason why they cannot be standardized. For the different pathogenic groups of AMV, Milbrath and McWhorter (10) originally suggested that tomato may be a useful host but later (11) recommended the use of lesion morphology on cowpea as the major basis of differentiation. Our results indicate that the systemic reactions on bean and cowpea would be a broader and perhaps less equivocal method of differentiation than lesion description.—Department of Botany and Plant Pathology, Purdue University, Lafayette; Department of Plant Pathology, University of Nebraska, Lincoln; and Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

LITERATURE CITED

1. Bancroft, J. B., and P. Kaesberg. 1958. Size and shape of alfalfa mosaic virus. Nature **181**: 720-721.
2. Bancroft, J. B., and P. Kaesberg. 1959. Macromolecular particles associated with alfalfa mosaic virus. (In manuscript.)
3. Black, L. M., and M. K. Brakke. 1954. Serological reactions of a plant virus transmitted by leaf-hoppers (Abstr.) Phytopathology **44**: 482.
4. Casals, J. 1957. Viruses: The versatile parasites I. The Arthropod-borne group of animal viruses. Trans. N. Y. Acad. Sci. **19**: 219-235.
5. Eckert, E. A., D. G. Sharp, Dorothy Beard, I. Green, and J. W. Beard. 1955. Virus of avian erthromyeloblastic leukosis. IX. Antigenic constitution and immunologic characterization. J. Natl. Cancer Inst., **16**: 593-643.
6. Henson, L., and S. Diachun. 1957. Effect of a strain of alfalfa mosaic virus on the yield of clonally propagated Atlantic alfalfa. (Abstr.) Phytopathology **47**: 15.
7. Kreitlow, K. W., O. J. Hunt, and H. L. Wilkins. 1957. The effect of virus infection on yield and chemical composition of Ladino clover. Phytopathology **47**: 390-394.
8. Malkiel, S. 1948. Immunochemical studies on tobacco mosaic virus. V. The serological relationship to the Aucuba and J14D1 strains. J. Immunol. **60**: 255-263.
9. Milbrath, J. A. 1952. Variations in potato calico caused by strains of alfalfa mosaic. (Abstr.) Phytopathology **42**: 516-517.
10. Milbrath, J. A., and F. P. McWhorter. 1953. The reaction of tomato varieties to various strains of alfalfa mosaic virus. (Abstr.) Phytopathology **43**: 479.
11. Milbrath, J. A., and F. P. McWhorter. 1954. Response of cowpea varieties to strains of alfalfa mosaic virus. (Abstr.) Phytopathology **44**: 498.
12. Moorhead, Ellen L. 1956. Serological studies of viruses infecting the cereal crops. I. A comparison of barley stripe mosaic virus and brome mosaic virus isolates by means of the complement-fixation techniques. Phytopathology **46**: 498-501.
13. Moorhead, Ellen L. Unpublished data.
14. Moorhead, Ellen L., and W. C. Price. 1953. A new serological test for tobacco mosaic virus. Phytopathology **43**: 73-77.
15. Oswald, J. W., A. Rozendaal, and J. P. H. van der Want. 1955. The alfalfa mosaic virus in the Netherlands, its effect on potato and a comparison with the potato aucuba mosaic virus. Proc. Second Conf. Potato Virus Diseases. p. 137-147. Lisse-Wageningen.
16. Rivers, T. M. 1948. Viral and rickettsial infections of man. Lippincott Co., Philadelphia, Pa., p. 477.
17. Salk, J. E., and Angela M. Laurent. 1952. The use of adjuvants in studies on influenza immunization. I. Measurements in monkeys of the dimensions of antigenicity of virus-mineral oil emulsions. J. Exptl. Med. **95**: 429-447.
18. Tall, M. G., W. C. Price, and K. Wertman. 1949. Differentiation of tobacco and tomato ringspot viruses by cross immunization and complement fixation. Phytopathology **39**: 288-299.
19. van Slogteren, D. H. M. 1955. Serological analysis of some plant viruses with the gel-diffusion method. Proc. Second Conf. Potato Virus Diseases. p. 45-50. Lisse-Wageningen.
20. Weaver, E. P., and W. C. Price. 1952. Differentiation of tobacco mosaic virus strains by complement fixation. Proc. Soc. Exptl. Biol. Med. **79**: 215-127.
21. Weimer, J. L. 1934. Studies on alfalfa mosaic. Phytopathology **24**: 239-247.
22. Zaitlin, M., A. M. Schechtman, J. G. Bald, and S. G. Wildman. 1954. Detection of virus in cattleya orchids by serological methods. Phytopathology **44**: 314-318.
23. Zaumeyer, W. J. 1953. Alfalfa yellow mosaic virus systemically infectious to beans. Phytopathology **43**: 38-42.

SURVIVAL OF PHYSIOLOGIC RACES OF PUCCINIA GRAMINIS VAR. TRITICI ON WHEAT NEAR BARBERRY BUSHES

C. W. Roane, E. C. Stakman, W. Q. Loegering, D. M. Stewart, and W. M. Watson

Respectively, Associate Plant Pathologist, Virginia Agricultural Experiment Station, Blacksburg, Virginia; Collaborator, Plant Pest Control Division, ARS, U. S. D. A., St. Paul, Minnesota; Senior Pathologist, Crops Research Division, ARS, U. S. D. A., Beltsville, Maryland; Pathologist, St. Paul, Minnesota; and formerly State Supervisor, Plant Pest Control Division, ARS, U. S. D. A., Roanoke, Virginia.

Accepted for publication June 23, 1959.

Cooperative investigations of the Virginia and Minnesota Agricultural Experiment Stations and the U. S. D. A., ARS, Plant Pest Control and Crop Research Divisions.

SUMMARY

Studies were made in Wythe County, Virginia, to obtain data on the relative persistence and increase of races of *Puccinia graminis* var. *tritici* in 4 wheat fields near rusted barberry bushes. During the 4 years 1950–53 inclusive, 42 races and subraces appeared in the fields. One, race 56, was found each year, 8 were found in each of 3 years, 8 others in each of 2 years, and 25 in 1 year only. The total virulence potential of these 42 races and subraces is so great that few commercial varieties of wheat in the United States would be resistant if all the races were to become widely prevalent. However, differences in survival ability were observed which may prevent the occurrence of such a situation. Race 56, for example, tended to increase and become prevalent in each of the 4 years, whereas race 15B showed no such tendency in the 3 years in which it was found. In this study, races 38 and 56 had especially good survival ability; 14, 15B, and 23 were intermediate; and races 23A, 48, 69, and 186A were poor in survival ability.

Berberis canadensis Mill., which is susceptible to *Puccinia graminis* Pers., is endemic in the Appalachian Highlands of Virginia and West Virginia, and stem rust limits wheat production where the bushes are abundant. Not only do many destructive local epidemics develop in areas infested with *B. canadensis*, but the reports of Stakman and others (3 through 15) show that physiologic races of *P. graminis* Pers. var. *tritici* Eriks. & E. Henn. are especially numerous in such areas and in certain areas of Pennsylvania and New York where *B. vulgaris* L. has escaped extensively from cultivation. In 1947, for example, all but 3 of the 15 races found in the entire United States were found in these 4 states. In addition, 43 races and subraces, many of which were not found elsewhere in North America (9), were isolated from 3 wheat fields adjacent to barberry bushes in Pennsylvania in 1949. The role of barberry in the production of numerous races is clear, but little is known about the relative ability of the different races to multiply and spread from the initial infections on susceptible wheat near the bushes.

The fact that many races are isolated from aecia or from uredia in the immediate vicinity of barberry bushes but not elsewhere suggests that they may have relatively low survival ability, either alone or in association with other races. Cassell (1) found strong indications that some races, notably race 38, were able to develop well at a wider range of temperature than others when cultured alone in the greenhouse. Watson (16) and Loegering (2) found that the relative ability of races to survive in mixtures on certain varieties of wheat that were apparently equally susceptible to the races in the mixture, differed with the combinations of races, the variety of wheat on which they were grown, and possibly with certain environmental factors. It therefore seemed desirable to study the spread and relative survival of races near barberry in nature; accordingly, intensive studies were made of collections of uredia obtained near barberries in Wythe County, Virginia, in the 4 successive years 1950–53.

Methods.—In each of the 4 years a different farm field of wheat was chosen for study. In 1950 the field was sown to Fulcaster wheat, in 1951 to Thorne, and in 1952 and 1953 to Vahart. The fields were selected because in each case stem rust was found developing on a single clump of *Berberis canadensis* at one edge of the field. The infection on these bushes originated from telia that had developed during the previous year on nearby cereals and grasses. In 1950 and 1952 the bushes were on the north side, in 1951 on the west, and in 1953 on the south.

Two sets of collections of uredia were made in each of these fields, along 7 axes radiating from the bushes through the fields (Fig. 1). The first collections were made near the barberry bushes shortly after uredia began to appear and before there was much chance for secondary spread. Collections were made at 10-ft. intervals along the 7 axes as far into the field as rust could be found. The uredia in these collections presumably resulted largely from infection by aeciospores from the adjacent barberry bushes. As the wheat neared maturity, a second set of collections was made at 100-ft. intervals along the 7 axes as far into the field as the rust extended or until the edge of the field was reached. Most of the rust in the field at this time undoubtedly resulted from infections by urediospores descended from the aeciospores that caused the primary infections, although a small amount may have resulted from direct infection by aeciospores. Although the collections were made according to a predetermined pattern, they probably were numerous enough to comprise adequate samples of the population of races.

The principal objective was to determine the relative prevalence of the more commonly occurring races rather than to determine the total number of races in the collections; therefore the following method was used in establishing cultures in the greenhouse. The collections were sent to the Cooperative Rust Laboratory at St. Paul, Minnesota, where seedling plants of Little Club wheat were inoculated from

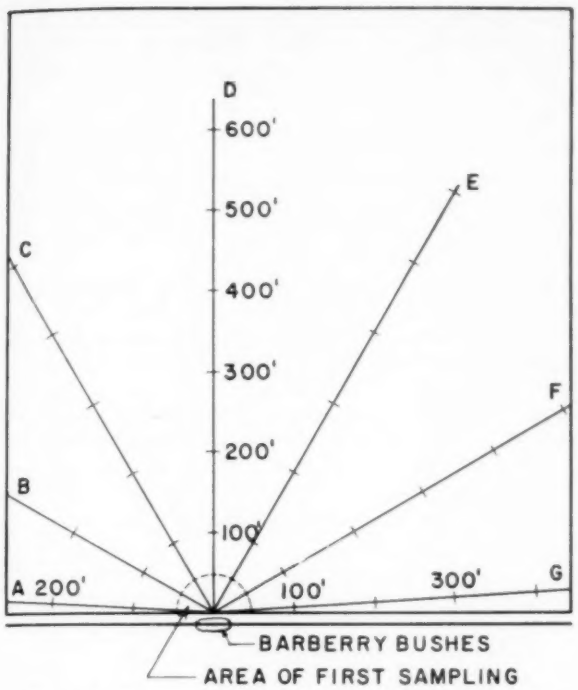


Fig. 1. Diagram of the 1950 wheat field, which illustrates the sampling plan for making uredial collections in the 4 different spreads of rust from barberry. Early-generation uredia were collected at 10-ft. intervals along axes A–G in "area of first sampling," and later-generation uredia were collected at 100-ft. intervals along axes A–G.

each collection and several single-pustule cultures were established from the resulting uredia. Race identifications were made of each single-pustule culture.[1] In only a few cases was more than one race obtained from each culture thus established.

The methods used were not entirely satisfactory for several reasons: 1) the races in the aecia on barberry were not determined; 2) studies were not made on the possible screening effect of wheat on aeciospore infections in the field; 3) the barberries should have been destroyed when the first set of collections were made to prevent further dissemination of aeciospores; 4) it is doubtful whether the number of single-pustule isolates was adequate in any year except 1950. Despite these inadequacies, the data permit certain conclusions about the races that spread from barberries and the relative survival ability of certain of these races.

Results.—The number of single-pustule cultures studied in the first and second sets of collections in 1950 was 120 and 105; in 1951, 59 and 34; in 1952, 43 and 26; and in 1953, 17 and 62 for a total of 466 cultures.

Races identified.—During the 4-year study 42 races and subraces were identified from the collections made in the 4 fields (Table 1). Undoubtedly, many more would have been found if the method of studying the collections had been especially designed for this purpose. Among the races and subraces identified there were many that are very similar, such as 23, 23A, 23B, 59, 59A, 59B, and 59C; 15 and 15B; 14 and 14A. The fact that so many recognizable races and subraces were isolated from the 4 fields demonstrates the role of barberries in unlocking the potential in *Puccinia graminis* var. *tritici* for recombination of genes for virulence on wheat. If all the races and subraces identified in this study were to become prevalent at one time, it would be very difficult to develop and maintain stem-rust-resistant wheat varieties.

Of the 42 races and subraces identified, only race 56 was identified one or more times in all 4 fields, 8 races and subraces (11, 14, 15B, 17, 59, 59A, 59B, and 69) were identified from 3 fields, and 8 other races (19, 23, 32, 33, 38, 48, 139, and 186) were identified in 2 different fields, whereas the other 25 races and subraces were found in only 1 field.

Relative survival of races.—The data obtained in this study with respect to the relative survival of the races making up the population spreading from the barberry may be analyzed in two ways. One is to compare the number of races in the two sets of collections obtained from each field, and the other is to compare the relative prevalence of the more commonly occurring races in the two sets of collections.

Theoretically, fewer races should be obtained proportionately in the second set of collections than in the first; hence, the ratio of cultures to races should be higher. In 1950 the ratio of cultures to races in the first set of collections was 5.0:1 and in the second set 5.8:1; in 1951 it was 4.5:1 and 4.3:1; in 1952 it was 3.3:1 and 2.4:1; and in 1953 it was 3.4:1 and 8.9:1. Thus, only in 1953 was there an appreciable difference between the ratios of races to cultures in the two sets of collections, but these data are probably inadequate since only 17 cultures were studied from the first set of collections.

The above results were to be expected since the methods used (single-pustule sub-cultures) were not designed to study the number of races but to determine the prevalence of the more commonly occurring races in a population. For the purposes of this study a race is considered to be common in a population if it is identified in more cultures than the ratio of cultures to races. Thus, in 1950, a race must have been identified 6 or more times in either set of collections; in 1951, 5 or more times in either set; in 1952, 4 or more times in the first set of collections and 3 or more times in the second; and in 1953, 4 or more times in the first set of collections and 9 or more times in the second. By these criteria, 11 races and subraces (14, 15B, 23, 23A, 38, 48, 56, 59, 59A, 69, and 186A) occurred commonly in one or more of the

[1] Race identifications were based on the key by E. C. Stakman, M. N. Levine, and W. Q. Loegering. Identification of physiologic races of *Puccinia graminis tritici*, U. S. D. A. Bureau of Entom. and Plant Quar. E-617. 1944. Since that key was published, the term subrace has been substituted for the term biotype. The latter term is now restricted to cultures derived from single urediospores or aeciospores.

populations. In 1950 the more commonly isolated races and subraces were 14, 23, 23A, 56, 59, 59A, and 186A; in 1951 they were 59, 59A, and 69; in 1952 they were 15B, 38, 48, 56, 59, and 59A; and in 1953 they were 38 and 56.

Race 14 was common in 1950 only and constituted 6.7% of the cultures in the first set of collections but only 0.8% in the second set. It was found also in 1951 and 1952 but showed no tendency to become more prevalent in the second set of collections. Thus, while race 14 spread from the barberry in 3 years, and in 1 year commonly, it appears to have little survival ability in relation to other races.

Race 15B was common in 1952 and was present in 1951 and 1953 also. In none of the 3 years did it show any tendency to increase or decrease appreciably. Thus this race spread from barberry but was barely able to maintain itself.

Race 38 was common in 1952 and 1953. In 1952 it constituted a relatively small percentage of the early cultures and showed a slight tendency to increase in prevalence later. In 1953 it was not found in the first set of collections but constituted 30.6% of the cultures in the second set.

Race 48 was common only in 1952, when it was found in the first set of collections but not in the second set; therefore, it apparently had low survival ability.

TABLE 1.—*Numbers of single-pustule cultures of physiologic races and subraces of Puccinia graminis tritici isolated from collections made early and late in 1950, 1951, 1952, and 1953 in fields of wheat to which rust had spread from barberry*

Race or subrace	1950		1951		1952		1953		No. years identified
	Early	Late	Early	Late	Early	Late	Early	Late	
9	–	1	–	–	–	–	–	–	1
11	–	–	–	1	–	1	2	2	3
14	8**	1	4	2	3	2	–	–	3
14A	–	2	–	–	–	–	–	–	1
15	–	–	4	4	–	–	–	–	1
15B	–	–	3	2	7*	6*	1	7	3
16	3	–	–	–	–	–	–	–	1
17	1	3	–	–	–	1	–	1	3
19	–	–	1	–	1	–	–	–	2
23	19*	11*	–	–	1	1	–	–	2
23A	25*	3	–	–	–	–	–	–	1
23B	1	–	–	–	–	–	–	–	1
27	–	–	–	–	–	–	1	–	1
27A	1	1	–	–	–	–	–	–	1
29	–	–	–	–	–	–	–	1	1
32	1	1	–	–	–	–	1	–	2
33	2	–	–	2	–	–	–	–	2
36	1	2	–	–	–	–	–	–	1
37	–	–	–	–	–	1	–	–	1
38	–	–	–	–	2	3*	–	19*	2
40	1	–	–	–	–	–	–	–	1
44	–	–	1	–	–	–	–	–	1
47	–	–	1	–	–	–	–	–	1
48	–	–	2	1	5*	–	–	–	2
52	–	1	–	–	–	–	–	–	1
56	1	55*	1	–	2	7*	12*	31*	4
59	11*	14*	7*	2	8*	–	–	–	3
59A	17*	2	23*	20*	8*	1	–	–	3
59B	2	–	2	–	2	2	–	–	3
59C	–	–	–	–	–	–	–	1	1
66	1	–	–	–	–	–	–	–	1
68	1	–	–	–	–	–	–	–	1
69	3	–	9*	–	1	–	–	–	3
69A	2	–	–	–	–	–	–	–	1
139	1	–	–	–	2	–	–	–	2
139A	3	2	–	–	–	–	–	–	1
152	–	–	1	–	–	–	–	–	1
162	–	1	–	–	–	–	–	–	1
180A	–	3	–	–	–	–	–	–	1
186	2	1	–	–	1	1	–	–	2
186A	10*	–	–	–	–	–	–	–	1
NR44[b]	3	1	–	–	–	–	–	–	1
Total isolates	120	105	59	34	43	26	17	62	
No. of races	24	18	13	8	13	11	5	7	
Isolates: races	5.0:1	5.8:1	4.5:1	4.3:1	3.3:1	2.4:1	3.4:1	8.9:1	

[a] A number marked with an asterisk indicates that the race occurred commonly. A race is considered to be common if it occurred more often than the ratio of cultures to races in a particular year and set of collections.

[b] NR signifies a "new race," one not described in the 1944 key to races.

Race 56 was common in 1950, 1952, and 1953. In 1950 it increased from 0.8% of the cultures in the first set of collections to 52.4% in the second; in 1952 it increased from 4.7 to 27.0%, whereas in 1953 it decreased slightly, from 70.6 to 50.0%. In the last year, however, because only 17 cultures were studied in the first set of collections, the apparent decrease cannot be considered significant.

Race 59 and subrace 59A were common in 1950, 1951, and 1952. In 1950 race 59 was about equally prevalent in both sets of collections, while subrace 59A constituted 14.2% of the cultures in the first set of collections but only 1.9% in the second. In 1951 the reverse was true, when race 59 decreased from 11.9% of the isolates in the first set of collections to 5.9% in the second, whereas subrace 59A more than held its own. In 1952, races 59 and 59A each constituted 18.6% of the cultures in the first set of collections but 0.0 and 3.9, respectively, in the second. Thus, it appears that differences in the climate or the wheat variety in the 3 years differentially affected the survival of this race and subrace.

Races 23 and 23A were common in 1950 and behaved in the same manner as 59 and 59A, respectively. These 4 races and subraces are very similar. The race 59 group produces a type "X" infection on the differential wheat variety Kubanka, whereas the race 23 group produces a type "4." On the differential variety Reliance races 23 and 59 produce type "0" infections but subraces 23A and 59A produce type "2" infections; otherwise, they produce the same infection types on the other 10 differential varieties.

Race 69 constituted 15.3% of the isolates in the first set of collections in 1951 but was not found in the second set. A few isolates of this race were also found in the first sets of collections in 1950 and 1952 but not at all in the second sets. It thus seems quite clear that race 69 has poor survival ability.

Subrace 186A constituted 8.3% of the cultures in the first set of collections in 1950 but was not found in the second set. This subrace was not found in any of the other years. This again is an example of a race with poor survival ability. It is also interesting that this subrace is differentiated from race 186 in the same manner as 59A is differentiated from 59. It will be noted that subraces 23A, 59A, and 186A all decreased sharply in prevalence in 1950.

Discussion.—Forty-two races and subraces were identified from uredial collections made in 4 fields of wheat that had been infected by aecia from barberries along one edge of the field. This demonstrates the great diversity in the race population of *Puccinia graminis* var. *tritici* that can result from recombination of factors for pathogenicity on the barberry. This extensive population of races was obtained in spite of the fact that the methods used in studying the collections were designed to keep this well-known fact from clouding the particular objective of the study, survival of the races. This diversity of races is important because it is known that all or nearly all commercial varieties of wheat would be susceptible to one or more of the races isolated.

There was a definite tendency for certain of the 42 races to predominate in the collections. Race 56 was found in all 4 fields, whereas 8 other races were found in 3 of the 4 fields. It is entirely possible that the phenotypes represented by these races resulted from combinations of dominant genes for pathogenicity; however, another explanation is that certain genes for pathogenicity were of more common occurrence in the telia that contributed to the development of aeciospores on the barberry. Actually, it is likely that both dominance and the more common occurrence of certain genes account for the development of certain races in several years or in relatively large numbers in any one year of the study.

It is evident from comparisons of the prevalence of common races in the first and second sets of collections that there are differences in the relative ability of the races produced on barberry to survive or spread on varieties of wheat that appear susceptible to all races. In the 4 years races 38 and 56 demonstrated special capability to survive, while races 23A, 48, 69, and 186A lacked this capability. Races 14, 15B, and 23 were intermediate in this respect. There is evidence that the ability of race 59 and subrace 59A to survive can be affected by the components of the environment such as climate and host variety.

The ability of certain races to survive and increase in mixed race populations is undoubtedly controlled by genetic factors, just as pathogenicity is controlled by genes. It is important, therefore, to distinguish between the screening effects of varieties and the ability of a race to survive and spread on susceptible hosts when associated with other races, which, for convenience, is designated as "survival ability." The total survival ability of a race in a large area depends on the number of varieties of wheat and other hosts on which it can develop, its ability to survive in mixed populations of races, and its ability to develop under different meteorologic conditions. Since the varieties of wheat in the present study were generally susceptible, the term survival ability, as used here, does not include host range and virulence, but ability to increase and spread in mixed populations of races on susceptible wheats under the meteorologic conditions that prevailed. For example, race 69 has poor ability to survive and race 56 has excellent survival ability; however, if these 2 races were cultured together on Vernal emmer, which is resistant to race 56 but susceptible to race 69, it is obvious that race 56 would quickly be screened from the mixture. Screening effect can be further illustrated by the differences between races 15B and 38. In the 1953 study, race 38 increased on Vahart from 0.0 to 30.6% of the cultures, whereas race 15B only held its own. In the same year, in the whole United States (15), race 15B composed 63.2% and race 38 only 7.4% of all uredial isolates. The high prevalence of 15B was not due to its greater "survival ability," which actually appears inferior to that of race 38, but to the known ability of race 15B to attack nearly all commercial varieties of wheat

then grown, whereas race 38 could attack only a few of them and was therefore screened out of the population to a considerable extent. It is evident that a distinction must be made between genes for virulence and those for ability to grow and reproduce rapidly. It is fortunate that some virulent races tend to become suppressed or eliminated when they occur in rust populations comprising numerous races such as those near barberry bushes.—Department of Plant Pathology and Physiology, Virginia Agricultural Experiment Station, Blacksburg; Division of Plant Pathology and Botany, University of Minnesota, St. Paul 1; and Plant Pest Control and Crops Research Branches, Agricultural Research Service, United States Department of Agriculture, Roanoke, Virginia, and Beltsville, Maryland.

LITERATURE CITED

1. Cassell, R. C. 1938. Factors affecting the distribution of physiologic races of Puccinia graminis tritici Erikss. and Henn. Unpublished Ph.D. Thesis, University of Minnesota.
2. Loegering, W. Q. 1951. Survival of races of wheat stem rust in mixtures. Phytopathology **41:** 56-65.
3. Stakman, E. C., R. U. Cotter, and W. Q. Loegering. 1943. Regional spread of wheat stem rust from barberry-infested areas of the Virginias in 1942. (Abstr.) Phytopathology **33:** 12.
4. Stakman, E. C., M. N. Levine, R. U. Cotter, and L. Hines 1934. Relation of barberry to the origin and persistence of physiologic forms of Puccinia graminis. J. Agr. Research **48:** 953-969.
5. Stakman, E. C., and W. Q. Loegering. 1942. Physiologic races of Puccinia graminis in the United States in 1941. U. S. Dept. Agr. Bur. Entomol. Plant Quarantine E-522-B. Mimeo. 11 p.
6. Stakman, E. C., and W. Q. Loegering. 1943. Physiologic races of Puccinia graminis in the United States in 1942. U. S. Dept. Agr., Bur. Entomol. Plant Quarantine E-522-C. Mimeo. 9 p.
7. Stakman, E. C., and W. Q. Loegering. 1945. Physiologic races of Puccinia graminis in the United States in 1943. U. S. Dept. Agr., Bur. Entomol. Plant Quarantine, Bur. Pl. Ind., Soils, and Agr. Eng., and Minn. Agr. Expt. Sta. Unnumbered Publication. Mimeo. 10 p.
8. Stakman, E. C., and W. Q. Loegering. 1948. Physiologic races of Puccinia graminis in the United States in 1944. U. S. Dept. Agr., Bur. Entomol. Plant Quarantine, Bur. Pl. Ind., Soils, and Agr. Eng., and Minn. Agr. Expt. Sta. Unnumbered Publication. Mimeo. 8 p.
9. Stakman, E. C., and W. Q. Loegering. 1950. Physiologic races of Puccinia graminis in the United States 1945-49. U. S. Dept. Agr., Bur. Entomol. Plant Quarantine, Bur. Pl. Ind., Soils, and Agr. Eng., and Minn. Agr. Expt. Sta. Unnumbered Publication. Mimeo. 20 p.
10. Stakman, E. C., and W. Q. Loegering. 1951. Physiologic races of Puccinia graminis in the United States in 1950. U. S. Dept. Agr., Bur. Entomol. Plant Quarantine, Bur. Pl. Ind., Soils, and Agr. Eng., and Minn. Agr. Expt. Sta. Unnumbered Publication. Mimeo. 16 p.
11. Stakman, E. C., W. Q. Loegering, R. C. Cassell, and L. Hines. 1943. Population trends of physiologic races of Puccinia graminis tritici in the United States for the period 1930 to 1941. Phytopathology **33:** 884-898.
12. Stakman, E. C., W. Q. Loegering, and R. U. Cotter. 1942. Physiologic races of Puccinia graminis in the United States in 1940. U. S. Dept. Agr., Bur. Entomol. Plant Quarantine E-522-A. Mimeo. 18 p.
13. Stakman, E. C., and D. M. Stewart. 1953. Physiologic races of Puccinia graminis in the United States in 1951. U. S. Dept. Agr., Bur. Entomol. Plant Quarantine, Bur. of Pl. Ind., Soils, and Agr. Eng. and Minn. Agr. Expt. Sta. Unnumbered Publication. Mimeo. 10 p.
14. Stakman, E. C., and D. M. Stewart. 1953. Physiologic races of Puccinia graminis in the United States in 1952. U. S. Dept. Agr., Bur. Entomol. Plant Quarantine, Bur. of Pl. Ind., Soils, and Agr. Eng. and Minn. Agr. Expt. Sta. Unnumbered Publication. Mimeo. 11 p.
15. Stakman, E. C., and D. M. Stewart. 1954. Physiologic races of Puccinia graminis in the United States in 1953. U. S. Dept. Agr., Plant Pest Control Branch, Field Crops Research Branch, and Minn. Agr. Expt. Sta. Unnumbered Publication. Mimeo 9 p.
16. Watson, I. A. 1942. The development of physiological races of Puccinia graminis tritici singly and in association with others. Proc. Linnean Soc. N. S. Wales **67:** 294-312.

INHERITANCE OF RESISTANCE TO FUSARIUM WILT IN UPLAND AND SEA ISLAND COTTONS AS COMPLICATED BY NEMATODES UNDER FIELD CONDITIONS

A. L. Smith and J. B. Dick

Pathologist and Agronomist, respectively, Crops Research Division, ARS, U. S. D. A., in cooperation with Agronomy and Soils Department, Alabama Polytechnic Institute Agricultural Experiment Station. Acknowledgment is made to J. O. Ware and Thomas Kerr for suggestions during the course of these investigations.

Accepted for publication June 23, 1959.

SUMMARY

Field studies on the inheritance of resistance to Fusarium wilt, *Fusarium oxysporum* f. *vasinfectum*, in Upland, *Gossypium hirsutum*, and Sea Island, *G. barbadense*, cottons were complicated by high populations of root-knot nematodes, *Meloidogyne incognita* var. *acrita*. Clear-cut genetic ratios for Fusarium resistance factors were obtained only by reducing the nematode population with low-level applications of nematocide. Wilt resistance in Upland cotton was controlled by a major dominant gene with modifying genes for Fusarium resistance and by additional genes determining root-knot nematode resistance. Fusarium resistance in Sea Island cotton was controlled by two dominant genes, which were additive in effect and gave a high degree of resistance. Only one of the genes in Sea Island cotton was sucessfully transferred to Upland cotton.

Introduction.—Fusarium wilt, caused by *Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyd. & Hans. has been the most serious disease of Upland, *Gossypium hirsutum* L., and Sea Island, *G. barbadense* L., cottons in the Southeastern States. Losses from wilt have been greatly reduced by growing resistant varieties. Data on the inheritance of resistance are important in making further improvement in commercial varieties. Investigation of the inheritance of resistance to Fusarium wilt was complicated by the relationship of nematodes to the disease. Nematode injury provides a means of entry for the pathogen into the vascular tissue of the host (11). Nematodes also increase host susceptibility in later stages of plant development, and a part of the wilt resistance in the more resistant commercial varieties can be attributed to their resistance to root-knot nematodes (10).

The 3 species of nematodes that have been implicated in the wilt-nematode complex are reniform nematode, *Rotylenchulus reniformis* Linford & Oliviera (9); sting nematode, *Belonolaimus gracilis* Steiner (3); and two types of root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood and *M. incognita* var. *acrita* Chitwood (8). The last-named is believed to be more widely distributed and more important in the wilt relationship than the other three combined. An abstract of this study on the inheritance of resistance to Fusarium wilt in Upland and Sea Island cottons has been published (13).

Fahmy (1) concluded that cotton wilt fungi from America, Egypt, and India were distinct varieties of *F. vasinfectum*. It appeared from his data (2) that the inheritance of wilt resistance in Egyptian varieties of *G. barbadense* was determined by one dominant major gene and one or more minor genes. Kulkarni (7) found that Fusarium resistance in *G. herbaceum* L. in India was determined monofactorially and that resistance was dominant. Kelkar et al (6), also using the Indian Fusarium, reported that resistance in *G. arboreum* L. was determined by 2 dominant, complementary genes and a third gene with inhibitory reactions.

In America, Jenkins et al (4) determined that resistance was dominant. In Upland, Hopi, Sea Island, and Egyptian crosses, the F_1 populations were as resistant as the more resistant parent. The F_1 crosses of Egyptian or Sea Island with susceptible Uplands were essentially immune. Smith and Ballard (12) reported resistant, susceptible, and segregating lines of Upland Empire among selfed lines developed on Fusarium-wilt-free soil. Their data suggested single-factor inheritance in Upland cotton. Jones (5) found that one major gene with absence of dominance accounted for resistance in the F_2 of a Delfos 425 × Half and Half cross. In F_3 lines, a deficiency of parental genotypes plus a continuous distribution of F_3 means was found. Jones suggested that modifying genes in addition to the major gene accounted for wilt resistance.

Materials and methods.—Studies were conducted in the field, where environmental factors could not be controlled, because greenhouse techniques for evaluating resistance to wilt in segregating populations had not been developed. The Upland parents used in this study were Cook 307 and Hurleys Rowden, hereinafter called Cook and Rowden. For many years Cook has been considered one of the most nematode- and wilt-resistant Upland cottons developed in the Southeast. Rowden is highly susceptible to both Fusarium wilt and root-knot nematodes. Selfing was begun in 1940 in Rowden and earlier in Cook. Selfs and crosses were made each year with Cook and Rowden, so that the parental lines, F_1, F_2, and backcrosses could be grown the following year. Three families from the same parental sources were established early in the study. Although, with continued selfing, Fusarium resistance was well fixed in Cook, some divergence in Fusarium or root-knot nematode resistance tended to make families 2 and 3 somewhat more Fusarium-wilt-susceptible than family 1.

Seabrook, a variety of Sea Island essentially immune to wilt, was crossed with Rowden as the susceptible parent. This interspecific cross was not grown beyond the F_2 generation, because of its wide segregation and production of some sterile plants. However, for study of the inheritance of resistance, this cross is considered satisfactory; Stephens, however, found that considerable selective elimination of the donor genotype may occur in backcrosses involving these 2 species (14).

The experimental material was planted at the Plant Breeding Unit, near Tallassee, Alabama, in an alluvial soil classified as Cahaba loamy fine sand and heavily infested with nematodes and the Fusarium wilt fungus. From 1949 to 1953, preplanting applications of 1–1½ ml of 40% ethylene dibromide fumigant per linear foot of row were made to control nematodes partially. Probably the only nematode involved in the wilt relationship was the cotton root-knot nematode, *M. incognita acrita*, although several other species parasitic on cotton were abundant. Plants showing wilt symptoms were removed at intervals through the growing season,

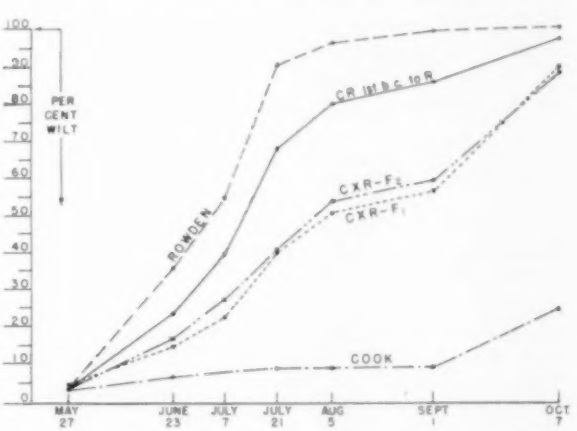


Fig. 1. Seasonal development of Fusarium wilt in an inheritance family of the cross Cook × Rowden. The soil was heavily infested with root-knot nematode, and wilt incidence was severe. 1947.

TABLE 1.—*Wilt inheritance in the Cook × Rowden cross, 1950 and 1952*

Parent or family	1950 Observed ratios (nw/w)[a]	1950 P	1952 Observed ratios (nw/w)[a]	1952 P
Cook	515/2		271/8	
Rowden	9/394		6/232	
Family 1:				
C×R, F_1	55/0		50/6	
C×R, F_2	216/70	.90–.80[b]	146/44	.70–.50[b]
(C×R)×R	15/12	.70–.50[c]	71/39	.01[c]
(C×R)×C	31/5		37/0	
Family 2:				
C×R, F_1	79/16		65/27	
C×R, F_2	406/213	<.01[b]	130/41	.80–.70[b]
(C×R)×R	77/133	<.01[c]	41/67	.05–.02[c]
(C×R)×C	42/9		33/1	
Family 3:				
C×R, F_1	104/53		53/14	
C×R, F_2	571/288	<.01[b]	160/35	.05–.02[b]
(C×R)×R	39/105	<.01[c]	29/37	.05–.02[c]
(C×R)×C	117/3		53/1	

[a] Ratios are number of non-wilted (nw) to number of wilted (w) plants.
[b] 3:1 ratio expected for one-factor hypothesis.
[c] 1:1 ratio expected for one-factor hypothesis.

from June 1 to September 1, except in 1947, when the last data were recorded on October 7. Families consisting of the two parents and F_1, F_2, and backcross progenies were randomized in complete blocks with 4–12 replications, the number varying with seed availability. The blocks were also randomized within the replication. Plots were 30 feet long and contained a maximum of about 40 plants. To give a large population, F_2 progenies were planted in several plots in each block. The parental lines were maintained and the crosses made at Auburn, where Fusarium wilt was of minor importance.

Experimental results.—*Inheritance of resistance in G. hirsutum.*—Fig. 1 shows the seasonal development of wilt in the Cook and Rowden parental lines and in the F_1, F_2, and the backcross to Rowden for 1947. The parental lines performed essentially as expected. The intermediate susceptibility of the Cook × Rowden F_1 suggested the absence of dominance. The paralleling of the F_1 and F_2 populations suggested that several genetic factors were involved. The Rowden backcross was only slightly more resistant than Rowden. Seed were not available for testing the backcross to Cook. There was no evidence of simple genetic ratios as reported by other workers (2, 6, 7). This lack of agreement was apparently the result of high nematode populations at this location.

The rapid increase in per cent wilt from September 1 to October 7, as shown in Fig. 1, may have been related to increased nematode populations and seasonal decline of the plants. However, in the absence of conclusive data or satisfactory explanation of this occurrence, September 1 was set as the last date for recording data in subsequent years.

In 1949, soil fumigation reduced nematode populations and the incidence of Fusarium wilt. Differentiation of the 2 parental lines was satisfactory. All Cook plants remained free of wilt symptoms, and all Rowden plants were killed by the disease.

Table 1 shows the results obtained with 3 families of Cook × Rowden in 1950 and 1952. Despite the application of soil fumigant, wilt incidence was too high to determine genetic ratios in 1951 and 1953. In all 3 families the behavior of the 2 parental lines was as expected, and the data were pooled. The F_1 crosses varied from fully resistant to about 34% susceptible. However, the data are considered as indicative of full dominance, particularly in view of the performance of Cook backcrosses, which were consistently resistant or showed only slight wilting. Three of the 6 F_2 populations gave P values (probability of Chi-squares) indicating that resistance was determined by a single gene pair with resistance dominant. The Rowden backcrosses gave P values slightly lower than did the F_2 populations, as a result of the introduction of additional root-knot susceptibility factors from Rowden. In comparison with family 1, the excessive numbers of wilted plants in families 2 and 3 in 1950 may be attributed to minor gene differences for Fusarium resistance between the Cook parents or possibly to gene differences in root-knot nematode resistance.

Seventy-eight F_3 plant populations selected at random from the F_2 of families 1 and 2 were tested in 1953. Cook showed no wilt, whereas Rowden showed 91.0 and 87.7%, respectively, in families 1 and 2.

TABLE 2.—*Wilt inheritance in the Seabrook × Rowden cross, 1950–52*

Parent variety of hybrid	1950 Observed ratio (nw/w)[a]	1950 P	1951 Observed ratio (nw/w)[a]	1951 P	1952 Observed ratio (nw/w)[a]	1952 P
Seabrook	76/0		90/0		40/0	
Rowden	0/64		0/95		1/88	
S×R, F_1	57/0		52/0		52/0	
S×R, F_2	112/13	.10–.05[b]	107/13	.05–.02[b]	57/2	.50–.30[b]
(S×R)×R	16/12	.05–.02[c]	65/35	.05–.02[c]	55/19	.90–.80[c]
(S×R)×S	29/0				15/0	

[a] Ratios are number of non-wilted (nw) to number of wilted (w) plants.
[b] 15:1 ratio expected for 2-factor hypothesis.
[c] 3:1 ratio expected for 2-factor hypothesis.

Thirteen per cent of the populations were as resistant as Cook, and 12% were as susceptible as Rowden. Eighteen of the 78 populations had less than 10% wilt. The remaining populations showed a continuous variation in percentage wilt. No clear separation could be made between homozygous or heterozygous resistant and susceptible classes. The continuous variation in the wilt percentages was attributed to the segregation of both Fusarium and root-knot resistance genes. Root-knot resistance is inherited recessively (11, 15) and the senior writer's unpublished data indicate that 3 or more genes are involved. Jones (5) suggested that modifying genes in addition to a major gene accounted for the continuous distribution of the wilt means and a deficiency of parental genotypes in F_2 lines. The writers' results also indicate the possibility of modifiers in the Cook × Rowden cross. However, the relation of nematodes to wilt incidence made it difficult to separate the effects of the major gene for Fusarium resistance from its modifiers and the indirect effect of multiple genes for nematode resistance.

Inheritance of resistance in G. barbadense.—Table 2 shows the results obtained from a Seabrook (Sea Island) × Rowden (Upland) interspecific cross in 1950 to 1952. Nematode populations and wilt incidence were too high in 1953 to obtain genetic data. In the Seabrook × Rowden cross, the data indicate that 2 dominant factors determine resistance to Fusarium. The behavior of the 2 parental lines and the F_1 was as expected. There was an excess of wilted plants in the F_2 and Rowden backcross progenies in both 1950 and 1951. This was attributed to the introduction of additional root-knot susceptibility factors from the Rowden parent, which was more susceptible to root-knot than Seabrook. Fusarium resistance in Seabrook was far superior to Upland resistance, suggesting that the two genes are additive in effect.

Transfer of Fusarium resistance from G. barbadense to G. hirsutum.—Transfer of the high Fusarium resistance of Seabrook to improve Upland commercial varieties was attempted by crossing Seabrook with Stoneville 2B and backcrossing twice to Stoneville 2B. Stoneville 2B was susceptible to Fusarium and slightly more tolerant to root-knot nematode than Rowden. Three F_4 lines of this hybrid were stabilized for Fusarium resistance, crossed with Rowden, and tested for wilt reaction in 1952. The results of this test (Table 3) indicate that resistances were determined by a single dominant factor. Thus, only one of the two resistance genes in *G. barbadense* was transferred in this cross. Although the trial was on a limited basis, it indicated that some difficulty may be encountered in transferring the 2 factors from *G. barbadense* to *G. hirsutum*.

Discussion.—This investigation emphasized the importance of the root-knot nematode relationship to Fusarium wilt in cotton and indicated the genetic basis on which breeding programs should be projected. Initially, the wilt reaction of Upland cotton suggested that multiple factors were involved in controlling resistance to Fusarium wilt. However, by reducing the root-knot nematode population with applications of ethylene dibromide, wilting was indirectly lowered to a level permitting identification of a single dominant factor for Fusarium resistance. The family × year interactions, which in 1951 and 1953 showed an excess of wilted plants over the expected, again emphasized that root-knot populations were an important factor in wilt incidence. Further, the introduction of root-knot-susceptible genes in Rowden crosses resulted in an excess of wilted plants beyond that expected in most 1950 and 1952 populations. The resistance of Cook to root-knot probably accounts for its greater wilt resistance, but minor genes for Fusarium resistance may be of importance.

Seabrook, Sea Island, was practically immune to Fusarium wilt, but it was more susceptible to root-knot nematode than Cook. This indicated that the two genes for Fusarium resistance in Seabrook gave greater resistance to wilt than the single Fusarium-resistance gene plus the root-knot-resistance genes in Cook.

The data obtained in the transfer of a Fusarium resistance gene from Sea Island to Upland may have a possible bearing on the origin of Fusarium resistance in *G. hirsutum*. In a personal communication, S. G. Stevens suggested that evolutionary information indicates that Fusarium resistance in *G. hirsutum* might have been derived from *G. barbadense*.

In a breeding program, the usual backcross approach is satisfactory where new characters are being transferred to established commercial wilt-resistant varieties. The transfer of wilt resistance to susceptible varietal types requires selfing and selection after each backcross to recover the recessive genes for root-knot resistance. Current commercial varieties have been developed largely from advanced generations of first crosses or from only one backcross followed by intensive selection on infested soils.—Department of Agronomy and Soils, Agricultural Experiment Station, Alabama Polytechnic Institute, Auburn.

TABLE 3.—*Wilt inheritance in the cross SSSS[a] × Rowden, 1952*

Parent or hybrid	Observed ratios nw/w[b]	P
SSSS	88/0	
Rowden	14/103	
SSSS × R, F_1	60/0	
SSSS × R, F_2	180/69	.05–.02[c]
[SSSS × R] × R	45/30	.10–.05[d]
[SSSS × R] × SSSS	45/3	

[a] SSSS refers to Seabrook × Stoneville 2B backcrossed twice to Stoneville 2B.
[b] Ratios are number of non-wilted (nw) to number of wilted (w) plants.
[c] 3:1 ratio expected for single-factor hypothesis.
[d] 1:1 ratio expected for single-factor hypothesis.

LITERATURE CITED

1. Fahmy, T. 1927. The Fusarium disease of cotton (wilt) and its control. Egypt. Ministry Agr., Tech. and Sci. Serv. Bull. No. 74.
2. Fahmy, T. 1934. Genetic basis of selection procedure with cotton wilt disease. Egypt. Ministry Agr. Tech. and Sci. Serv. Bull. 128.
3. Holdeman, Q. L., and T. W. Graham. 1954. Effect of the sting nematode on expression of Fusarium wilt in cotton. Phytopathology. **44:** 683-685.
4. Jenkins, W. H., E. E. Hall, and J. O. Ware. 1939. Notes on inheritance of Fusarium wilt. S. C. Agr. Expt. Sta. 52nd Ann. Rept. 119-121.
5. Jones, J. E. 1953. The influence of modifying genes on Fusarium wilt resistance in upland cotton (Abstr.) Am. Soc. Agron. Abstracts. p. 90-91.
6. Kelkar, S. G., S. P. Chowdhari, and N. B. Hiremath. 1947. Inheritance of Fusarium resistance in Indian cottons. Indian Central Cotton Comm., 3rd Conf. on cotton growing problems in India. Bombay, p. 125-162.
7. Kulkarni, G. S. 1934. Studies in the wilt disease of cotton in the Bombay Presidency. Indian J. Agr. Sci. **4:** 976-1045.
8. Martin, W. J., L. D. Newsom, and J. E. Jones. 1956. Relationship of nematodes to the development of Fusarium wilt in cotton. Phytopathology. **46:** 285-289.
9. Neal, D. C. 1954. The reniform nematode and its relationship to the incidence of Fusarium wilt of cotton at Baton Rouge, La. Phytopathology. **44:** 447-450.
10. Smith, A. L. 1953. Fusarium and nematodes on cotton. Yearbook Agr. U. S. Dept. Agr., p. 292-298.
11. Smith, A. L. 1954. Problems on breeding cotton for resistance to nematodes. Plant Disease Rept. Suppl. **227:** 90-91.
12. Smith, A. L., and W. W. Ballard. 1947. Wilt resistance in Empire cotton. (Abstr.) Phytopathology. **37:** 436-437.
13. Smith, A. L., and J. B. Dick. 1952. The inheritance of resistance to Fusarium wilt in upland and Sea Island Cotton. (Abstr.) Phytopathology **42:** 287-288.
14. Stephens, S. G. 1949. The cytogenetics of speciation in Gossypium. I. Selective elimination of the donor parent genotype in interspecific backcrosses. Genetics **34:** 627-637.
15. Wiles, A. B. 1957. Resistance to root-knot nematode in cotton. (Abstr.) Phytopathology. **47:** 37.

INOCULATION OF SLASH PINE SEEDLINGS WITH CRONARTIUM FUSIFORME

F. F. Jewell

Plant Pathologist, Southern Institute of Forest Genetics, Southern Forest Experiment Station, Forest Service, U. S. D. A., Gulfport, Mississippi.

Accepted for publication June 25, 1959.

SUMMARY

Techniques have been developed for inoculating large numbers of pine seedlings with fusiform rust. Cotyledonary slash pine seedlings in nursery beds were inoculated by placing telia-bearing oak leaves over them and maintaining high humidity for 72 hours; infection was through the needles, with a close correlation between needle spotting and later-developing stem galls. One-year-old seedlings were inoculated by wrapping the new growth in telia-bearing oak leaves and maintaining high humidity for 72 hours, and also by inserting telia into new stem tissue; infection was directly into the stem by both methods.

Although fusiform rust (*Cronartium fusiforme* (A. & K.) Hedgc. & Hunt) is probably the most serious disease of slash pine (*Pinus elliottii* var. *elliottii* Little & Dorman) throughout its natural range, little information is available on early symptomatology and inoculation techniques suitable for large-scale experiments—as in testing for rust resistance in a genetics program. Research on these subjects is reported here.

Methods and materials.—The experimental plants were nursery-grown slash pine seedlings of two ages—3 to 6 weeks, and 1 year, the ages at which progenies from breeding programs will probably be first tested for rust resistance. Inoculum consisted of naturally infected fresh oak leaves (mostly *Quercus nigra* L.) bearing sporidia-producing telia.

Inoculation of seedling-stage plants.—During the early spring of 1956, local slash pine bulk seed was sown at the rate of 60 seeds per sq. ft. in six 4×4-ft. nursery beds in southern Mississippi. The seedlings were inoculated when about 30 days old, i.e., when cotyledons were well developed. Inoculation chambers were single- or double-frame canvas tents fitted over the individual beds (Fig. 1-AB). Small oak branches were inserted in the soil so that the attached inoculum-bearing leaves formed a canopy over the seedlings (Fig. 1-A).

Humidity near the saturation point and temperatures of 16–26°C for a minimum of 18 hours have been reported necessary for *C. fusiforme* sporidial production and infection of pine (2). In attempts to produce such conditions, a water mist was applied intermittently over the seedlings and inoculum in the tents during the first 8 hours of the experiments. Water was also constantly sprayed on the outside of the single tent cover and between the layers of the double tent. A large burlap cover was necessary around the single-frame tent to prevent wind-blowing of the external water. Fig. 1-A,B shows details of the spray system. The tents were kept over the seedlings for 72 hours. Each tent was used to inoculate 2 beds, with 2 additional beds left untreated as checks. Four weeks after inoculation the bed densities were reduced to about 30 plants per sq. ft. to allow more growing space per plant.

In 1957 two 4×4-ft. beds of 4–6-week-old slash pine seedlings from bulk seed were thinned to about 20–30 seedlings per sq. ft. after inoculation under the single tent. A third bed was left untreated.

All seedlings were lifted when about 9 months old, and rust infection was tallied on the basis of the formation of typical fusiform galls on the lower stems.

Inoculation of yearling plants.—Three methods of inoculation were applied to individual slash pine seedlings potted after 1 year in the nursery bed.

1) *Oak-leaf wrap* (Fig. 1-C).—The new flush of growth on 80 plants was wrapped in infected oak leaves. On 40 of the plants the inoculum was wrapped in absorbent cotton kept moist by hand spraying. Seedlings were stored in a lath-house, the oak leaves and cotton being removed after 72 hours. The remaining 40 plants were placed for 72 hours in a large moist chamber (Fig. 1-D) over which water was sprayed constantly. The oak leaves were then taken off and the plants removed to the lath-house.

2) *Sporidial spray.*—A suspension of sporidia, made by soaking 5 telia/ml of distilled water, was sprayed with an atomizer on the previously moistened new flush of growth (including needles) of 10 slash pines, which were then placed in the lath-house for incubation and observation. No count or viability check was made of sporidia in the suspension.

3) *Telial insertion.*—Under a dissecting microscope, sporidia-producing telial columns or small telial-bearing pieces of oak leaves were inserted into slits and V-shaped cuts, respectively, in new and old stem portions of 18 plants. On three similar plants, slits were made in the new stem growth but no inoculum was introduced. This method is similar to that used by Hedgcock and Siggers (1).

4) *Checks.*—Twenty potted slash pines were placed in the lath-house for determining how much infection occurred naturally.

Results.—About 4–6 weeks after inoculation, small red to green-red mottled spots and bands with and without definite margins were noted on the cotyledons of a majority of the inoculated seedlings (Fig. 2-C). Microscopic examination of hand sections through a large number of these spots revealed typical *Cronartium* mycelium.

Table 1 shows the amount of needle spotting at 4–6 weeks and the number of stem galls at 8 months after inoculation. Typical galls are shown in Fig. 2-E. There was no significant difference in the effectiveness of the double and single tents. Both resulted in a high degree of infection.

The results of the various methods used on the 1-0 slash pine stock are given in Table 2. The most suc-

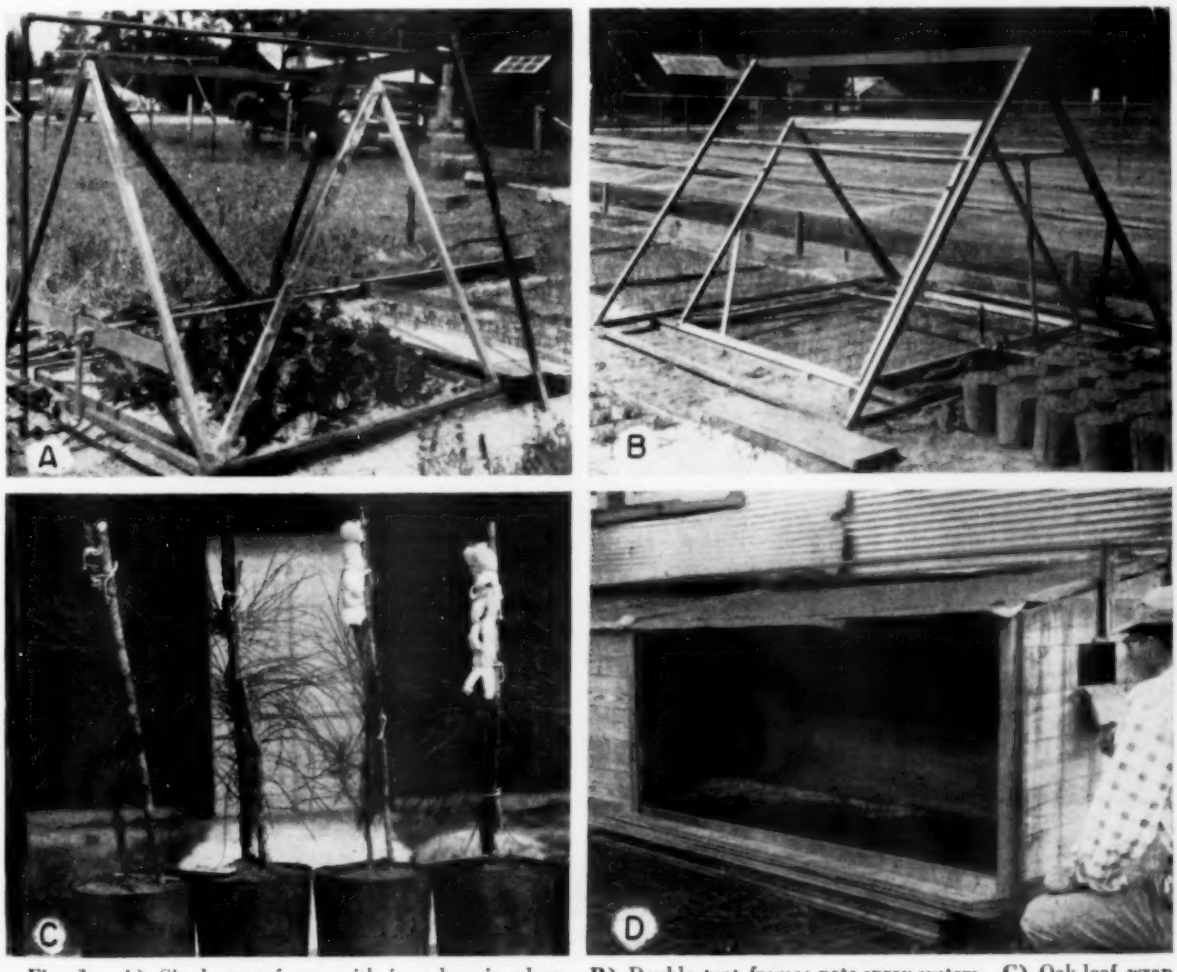


Fig. 1. A) Single tent frame with inoculum in place. B) Double tent frame; note spray system. C) Oak-leaf wrap with 1-0 stock with and without cotton covering. D) Chamber used for inoculating 1-0 stock.

TABLE 1.—*Number of surviving slash pine seedlings and percent of plants with needle spots and rust galls after inoculation with Cronartium fusiforme*

Inoculation method and date	Original number of plants inoculated	4–6 weeks after inoculation		8 months after inoculation	
		Number plants surviving	% plants galled	Number plants surviving	% plants galled
Double tent					
April 9–12, 1956	685	477	98	442	87
April 20–23, 1956	647	464	69	459	71
Check	711	474	5	456	2
Single tent					
April 10–13, 1956	792	485	89	472	74
April 20–23, 1956	524	382	85	376	86
Check	807	491	5	482	3
April 19–22, 1957	585	325	89	306	82
May 3–6, 1957	436	233	10	233	33
Check	643	352	3	350	5

cessful method was wrapping new shoot growth of the plants with oak leaves. With this method, symptoms of infection appeared about 8–10 weeks after inoculation. These symptoms, which occurred on the stem only, were purplish to blue-purple blotches with indefinite margins that varied from a few millimeters in length and diameter to 75 mm long. Rarely did these symptoms occur around the entire circumference of the stem.

Characteristic stem swellings became evident 8–9 months after inoculation, and all were in the stem area originally wrapped. The swellings varied somewhat in size and shape. In some pines, several infections coalesced to form a large swelling (Fig. 2-A) while others had galls considered typical of single infections (Fig. 2-B). The successful infections from telial insertions occurred only on the new shoot growth. The infections became obvious at the same time as those from the wrapping technique, but appeared slightly more localized (Fig. 2-D). None of

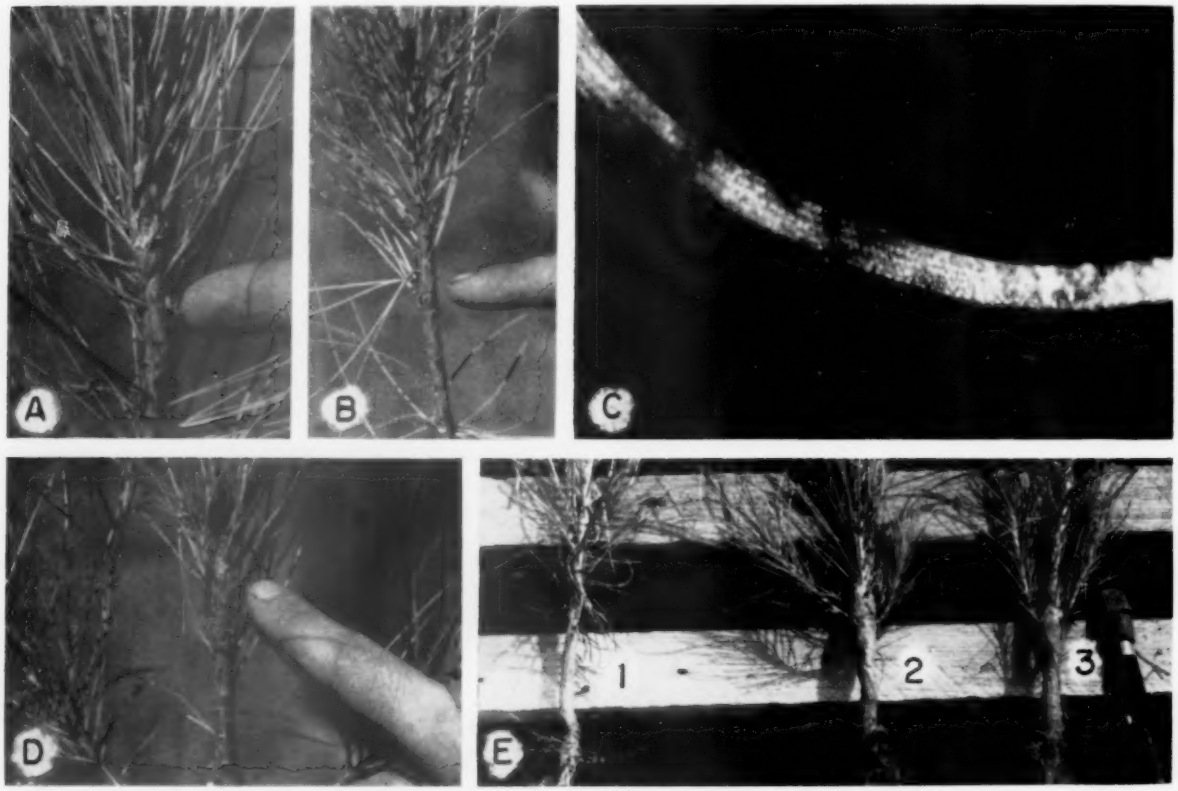


Fig. 2. **A)** Multiple infection on 1-0 slash pine stem. **B)** Single infection on 1-0 slash pine stem. **C)** Slash pine cotyledon showing symptoms of rust infection 6 weeks after inoculation. **D)** Infection on slash pine resulting from insertion of telial material. **E)** Nine-month-old slash pine seedlings; (1) rust-free control; (2, 3) plants showing galls 8 months after inoculation.

TABLE 2.—*Results of inoculating 1-0 potted slash pine seedlings with Cronartium fusiforme*

Inoculation method	No. of plants	No. infected
Oak-leaf wrap		
No cotton	40	21
With cotton	40	30
Sporidial spray	10	0
Telial insertion	9	3
Telia-bearing piece of oak leaf inserted	3	1
Check	20	0

the check plants developed rust infections.

Discussion.—The tests show that large numbers of slash pines can be simultaneously inoculated with fusiform rust under artificial conditions, and will show infections within 12 months. The inoculations were most successful during the period of peak telial production in nature, during April in 1956 and 1957. The one bed inoculated in May, with inoculum slightly past maturity, had less infection.

Results with 1-0 nursery stock show that such material can be artificially inoculated with rust under certain conditions. Since each plant was handled individually, the method would be laborious with large lots but might be useful for testing small groups of seedlings, i.e., reinoculating individuals still rust-free after nursery-bed inoculations.

The correlation between gall formation and earlier needle spotting (Table 1) is further confirmation of the early symptoms of fusiform rust on slash pine. These symptoms can possibly serve as an initial measure of resistance or susceptibility of pine progenies in future testing work. The presence of foliar symptoms on a particular progeny with no stem infection resulting would be a stronger indication of resistance than where no such symptoms were present. It would be known that the plant was not an escape but that infection had occurred without progress of the fungus to the stem. However, gall-formation failure might be due to some physiological or anatomical influence of the host in preventing mycelial spread in the stem, or death and abscission of infected needles before the mycelium had progressed to the stem.

With potted 1-0 pines, needle spots were never observed, even on very young secondary needles that were 4–28 cm long at the time of inoculation. The symptoms appeared only on the stem—indicating direct penetration through the young epidermis of the shoot rather than through the needle. The opposite was true for the young nursery plants; infection spots appeared on the needles (cotyledons) and rarely on the stem.

LITERATURE CITED

1. HEDGCOCK, G. G., AND P. V. SIGGERS. 1949. A comparison of the pine-oak rusts. U. S. Dept. Agr. Tech. Bull. 978, 30 p.
2. SIGGERS, P. V. 1947. Temperature requirements for germination of spores of *Cronartium fusiforme*. Phytopathology **37**: 855-864.

DIFFERENTIAL LONGEVITY OF TELIOSPORES OF PATHOGENIC RACES OF TILLETIA CARIES AND T. FOETIDA

E. L. Kendrick and C. S. Holton

Pathologists, Crops Research Division, ARS, U. S. D. A., Pullman, Washington.

Accepted for publication June 26, 1959.

Cooperative investigations of the Crops Research Division, ARS, U. S. D. A., and the Agricultural Experiment Stations of Idaho, Oregon, and Washington. Scientific Paper No. 1833, Washington Agricultural Experiment Stations, Pullman.

SUMMARY

Germination tests were made of new and old teliospores of 11 races of *Tilletia caries* and 7 races of *T. foetida*. The old spore material was stored at room temperature in powder form and as bunt balls and tested after 18 and 22 years' storage. The rate of germination was slower in the old spores of all races, with the decreased rate being greater in some races than in others. Although the spores of all races were still viable after 18 years, differences in longevity were evident among races. Further aging, up to 22 years, revealed highly significant differences in spore longevity among races. Generally, there was little or no difference in spore longevity, whether the spores were stored in ball or powder form. Viable 22-year-old spores were infective only if germinated before inoculation of the seed.

The longevity of the teliospores of smut fungi is important to survival of the species and to control of the diseases they cause. Smut spore longevity can range from a few months, as in the floral-infecting species *Ustilago nuda* (Jens.) Rostr., to many years, as in the seedling-infecting species *Tilletia caries* (DC.) Tul. and *T. foetida* (Wallr.) Liro. Thus, the last two may be classed as long-lived species, although the limits of their inherent potential spore longevity have not been determined by systematic study. Furthermore, the expression of this inherent longevity potential might depend on specific storage conditions, such as temperature and humidity. Only limited attention has been given to these and other factors in longevity studies with *T. caries* and *T. foetida* thus far reported.

Spores of *T. caries* known to be 12 years old were germinated by Woolman and Humphrey (9), and 7-year-old spores of both species were germinated by Lobik and Dahlstrem (4). Fischer (2) observed the viability of spores from 18-year-old herbarium specimens of *T. caries* and of spores from 25-year-old herbarium specimens of *T. foetida*. The respective

TABLE 1.—*Rates and percentages of germination of new and old spores of 10 races of Tilletia caries and T. foetida stored in ball form*

	Spore ages, and days required for germination shown					
	New (1–3 years)		Old (18 years)		Old (22 years)	
Race[a]	Days	%	Days	%	Days	%
T-1	6	75	15	50	25	50
T-5	5	75	15	75	30	5
T-7	5	75	9	75	17	50
T-9	6	75	13	75	21	70
T-10	9	75	19	60	25	60
T-11	9	75	31	Tr	37	0
L-1	5	75	15	75	30	20
L-4	8	75	15	60	30	50
L-7	5	70	24	12	37	0
L-8	5	75	11	75	21	50

[a] Of 8 races also studied, but not listed in the table, races T-2, T-3, T-4, and T-6 behaved like T-1; T-8 behaved like T-7; L-2 behaved like L-1; and L-3 and L-5 behaved like L-8.

germination periods were 12 and 21 days, significantly longer than required for the germination of fresh spores of these two species. Olgyay (6) suggested that the spore-longevity potential of *Tilletia* spp. is not related to age alone but may also be related to the source (presumably race affiliation) and to whether the spores are stored in powder form or as bunt balls. According to Rabien (7), the rate and amount of teliospore germination of both species decrease progressively, beginning the second year after spore maturity. Ajroldi (1) observed that viability reached its maximum in the second year and remained high for 5 years.

The incidental nature of these above studies, and the differences in materials and methods used, together with certain contradictory aspects of the results, pointed up the need for a systematic study designed to take into account the various factors that might influence spore longevity in these species. This is a report on such a study, initiated in 1936, on which two previous reports have been made (3, 5).

Materials and methods.—The material used in this study on longevity represented new and old spores of 11 races of *T. caries* (T-races) and 7 races of *T. foetida* (L-races) (8). The new spore material of each race was composed of a composite of spore balls, ranging from 1 to 3 years old, stored in coffee cans at room temperature. This material represents current stocks of spore material used for pathogenicity studies. The old spore material had been collected and prepared in 1936. It was composed of spore balls and powdered spores of each race, stored separately in capped glass jars at room temperature. Germination was tested after 11 years (5), 18 years (3), and now after 22 years. These old spores had exhibited high rates of viability (75–90% germination in 5–9 days) in the fresh stage, when they were first prepared for storage, thus establishing a common base for measuring spore longevity.

Tests for spore germination were made on 3% water agar in Petri plates. Spores stored in powder form were scattered onto the agar from a sterile spatula. Those in ball form were freed by crushing with forceps, after the spore ball had been surface-sterilized by dipping it in alcohol and flaming. Several random samples of either spore balls or spore powder were taken from each spore lot (one spore lot per race) as a check against possible variability in rate of germination within the same lot of spores. The test with each random sample was replicated twice, the total replications for each spore lot being at least 8. The spore-bearing agar plates were incubated at 15°C.

Observations on spore germination were made at 24-hour intervals, beginning on the fourth day of incubation. The percentage of germination was based upon the average of counts of 100 spores made in at least 3 replications. Germination percentages above 75 could not be determined, because at this stage the intermingling of colonies prevented accurate counting. Germination percentages below 75 indicate maximum germination.

Comparative pathogenicity tests with new and old

TABLE 2.—*Rates and percentages of germination and infection with spores of 10 races of T. caries and T. foetida stored as smut balls and as powdered spores.*

	Spore ages, storage form, and days required for germination shown								
	18 years				22 years				
	Ball		Powder		Ball		Powder		
Race[a]	Days	%	Days	%	Days	%	Days	%	Infection %
T-2	10	75	10	75	25	60	25	55	12
T-4	13	70	17	60	30	50	37	2	65
T-5	15	75	17	50	30	5	30	3	5
T-7	9	75	11	75	17	50	21	50	71
T-10	19	60	19	60	25	60	25	50	52
T-11	31	Tr	24	8	37	0	37	0	0
L-1	15	75	18	50	30	20	30	3	50
L-4	15	60	15	60	30	50	37	50	0
L-7	24	12	24	35	37	Tr	37	Tr	6
L-8	11	75	15	50	21	50	30	40	36

[a] Of 8 races also studied, but not listed in the table, races T-1, T-3, and T-6 behaved like T-2; T-8 behaved like T-7; T-9 behaved like T-10; L-2 and L-5 behaved like L-1; and L-3 behaved like L-4.

spores were also made as a further study of spore longevity and its practical implications. Seed of Red Bobs spring wheat was inoculated with spores, planted in moist sand, and incubated 14 days at 10°C. The seedlings were then transplanted and grown to maturity in the greenhouse. To compensate for any delay in germination of the old spores that might allow the host seedling to develop beyond the susceptible stage before spore germination, another pathogenicity test was made by inoculating the seed with pre-germinated spores. This was done by applying directly to the seed a suspension of germinated spores, or ungerminated spores that had been incubated for 37 days. Infection percentages in all pathogenicity tests were based on total and smutted heads in each row.

Results and discussion.—For the sake of clarity of tables, the results of only 10 representative races of the 18 studied are presented in Tables 1 and 2. Races not in the tables but behaving similarly to races listed are presented in footnotes to the tables. The general results and discussion concern all 18 races.

The rate of germination of the old spores of all races was slower than that of new spores. The 18-year-old spores required about 2-3 times as long to germinate as the new spores, and the 22-year-old spores required up to twice as long to germinate as the 18-year-old spores. The decrease in rate of germination of the old spores was greater in some races than in others. For example, the new spores of T-5 and T-7 germinated at the same rate, whereas the old spores of T-7 germinated more rapidly than the old spores of T-5 (Table 1). Other races (T-10 and T-11, L-7 and L-8) exhibited similar differences.

Some of the races also differed in spore longevity. Although some spores of all 18 races were still viable after 18 years, those of T-11 showed only a trace and those of L-7 only 12% of viability at this age (Table 1). The remainder of the races exhibited little or no decrease in germination percentage after 18 years. Further aging, up to 22 years, revealed highly significant differences in spore longevity among races. Some of them (T-1, T-9, T-10) had about the same percentage viability as exhibited after 18 years, but others (T-7, L-1, L-4, L-8) were somewhat less viable at the end of 22 years. The most striking reduction in percentage viability was exhibited by T-5, which decreased from 75% viability after 18 years to 5% after 22 years. There was an apparent complete and nearly complete loss of viability, respectively, by races T-11 and L-7, which exhibited little or no spore germination in this test after 37 days' incubation.

The results of the study on comparative longevity of 18- and 22-year-old spores of different pathogenic races when stored as bunt balls and as powdered spores are presented in Table 2. In general, the longevity of spores stored in ball form was only slightly better than that of spores stored in powder form. Some races, such as T-2, T-7, T-10, and L-4, showed no difference in viability of spores stored in ball or powder form for 18 years, and little or no difference after 22 years. Slightly greater longevity of 18-year-old spores stored in ball form was exhibited by some races (T-4, T-5, L-1, L-8). After 22 years this difference was more pronounced in two of these (T-4, L-1), about the same in one (L-8), and all but lost in the fourth race (T-5). Only 2 races (T-11, L-7) exhibited an apparently greater longevity of spores in powder form, both in the case of 18-year-old spores (Table 2).

Tests for pathogenicity of the 22-year-old spores produced infection percentages shown in Table 2, last column. These results were obtained from inoculations with germinated spores. No infection was obtained from inoculation of the seed with ungerminated spores, because of the slow rate of germination, which allowed the host seedling to develop beyond the point of susceptibility prior to spore germination. Thus, although some 22-year-old spores were viable, they were innocuous as seed-borne inoculum. Nevertheless, these spores, except those of L-4, had retained their infectivity, as evidenced by the infection produced by germinated spores (Table 2).

This study indicates pronounced differences in the spore longevity of different pathogenic races of *T. caries* and *T. foetida*. The spores of some races become nonviable after 22 years, while others retain a high degree of viability at this age. Aging of spores greatly decreases their speed of germination. This decrease is greater in some races than in others in the same period. Decreased rates of spore germination of these races were observed and reported first at 11 years (5). The present study shows that the germination rate of these same races continues to decrease with increasing age of the spores. It further shows that germination can be so slow that viable spores are innocuous as seed-borne inoculum. Thus, age of spores is an important factor in pathogenicity tests where infection depends on coordination between spore germination and host development in the susceptible stage.—Regional Smut Research Laboratory, Agricultural Experiment Station, Pullman, Washington.

LITERATURE CITED

1. AJROLDI, P. 1940. Nuovo contributo allo studio biologico delle 'Tilletia'. Riv. patol. vegetale **30:** 149-157. (Abstr. in Rev. Appl. Mycol. **19:** 525. 1940.)
2. FISCHER, G. W. 1936. The longevity of smut spores in herbarium specimens. Phytopathology **26:** 1118-1127.
3. KENDRICK, E. L. 1954. Differential longevity in spores of physiologic races of Tilletia caries. (Abstr.) Phytopathology **44:** 494.
4. LOBIK, V. I., AND A. F. DAHLSTREM. 1936. [Improvement of methods for the germination of wheat bunt spores in the laboratory.] Summ. Sci. Res. Wk. Inst. Pl. Protect. (U.S.S.R.) Leningr., 1935, p. 177-178. (Russian; trans. title) (Abstr. in Rev. Appl. Mycol. **15:** 786-787).
5. LOWTHER, C. V. 1950. Chlamydospore germination in physiologic races of Tilletia caries and Tilletia foetida. Phytopathology **40:** 590-603.
6. OLGYAY, M. 1935. [Untersuchungen über das Keimen und de Infektionsverhaltnisse der Steinbrandsporen (Tilletia foetans and T. tritici)]. (Hungarian with German summary.) Botanikai Közlemenyek **32:** 52-74.

7. Rabien, H. 1927. Ueber Keimungs- und Infektionsbedingungen von Tilletia tritici. Arb. biol. Reichsanstalt. Land-Forstwirtsch. **15**: 297-353. (Abstr. in Rev. Appl. Mycol. **7**: 83-86. 1928.)
8. Rodenhiser, H. A., and C. S. Holton. 1937. Physiologic races of Tilletia tritici and T. levis. J. Agr. Research **55**: 483-496.
9. Woolman, H. M., and H. B. Humphrey. 1924. Studies in the physiology and control of bunt, or stinking smut, of wheat. U. S. Dept. Agr. Bull. 1239. 30 p.

A NEW SOURCE OF RESISTANCE TO SPINACH BLIGHT

R. E. Webb, Bruce A. Perry, Henry A. Jones, and D. M. McLean

Respectively: Crops Research Division, ARS, U. S. D. A., Beltsville, Maryland; Winter Garden Substation No. 19, Texas Agricultural Experiment Station, Crystal City, Texas; Collaborator; and Crops Research Division, ARS, U. S. D. A., Weslaco, Texas.

Accepted for publication June 26, 1959.

SUMMARY

A spinach variety recently introduced from Belgium was found highly resistant to spinach blight at temperatures up to 28°C. Inoculations of progenies of crosses of resistant P.I. 179590 and the susceptible variety Bloomsdale indicated that resistance is controlled by a single dominant gene. Assays of resistance of P.I. 179590 and Virginia Savoy in relation to strains of cucumber virus 1 indicate that there are strains of the virus to which there is little or no resistance at present.

Spinach blight, caused by strains of the cucumber virus 1, is often a production problem to growers in many areas (4, 8). Epidemics of the disease are usually associated with abnormally high temperatures during the growing season. Warm periods during late fall and early spring increase the activity of the virus vectors and increase rapidity of expression of the disease. Resistant varieties such as Virginia Savoy and Old Dominion have minimized the importance of spinach blight during most seasons in areas to which they are adapted. Other areas are in need of adapted varieties and hybrids highly resistant to blight.

The introduction of high-yielding spinach hybrids (4, 5, 6) resistant to blue mold incited by *Peronospora effusa* (Grev. ex. Desm.) Ces. increased interest in spinach production, particularly along the eastern seaboard and in the southern states. These areas are often plagued with spinach blight, and resistance greater than the type (7) in blue-mold-resistant varieties now available to breeders would be decidedly advantageous in developing adapted disease-resistant hybrids.

In 1955, a routine screening of spinach lines introduced from foreign countries was begun in search of a high level of resistance to spinach blight. An introduction from Belgium, P.I. 179590,[1] was found to be heterozygous for resistance to a spinach blight strain of cucumber virus 1. Preliminary trials indicated that the level of resistance inherent in P.I. 179590 is stable at temperatures at which the resistance possessed by Virginia Savoy and related varieties and hybrids breaks down (7). Through sibmating and backcrossing, a selection of P.I. 179590 homozygous for resistance to the spinach blight strain of cucumber virus 1 was obtained. The relative levels of resistance possessed by this selection at various temperatures were compared with those in Virginia Savoy at the same temperatures. Since strains of cucumber virus 1 highly infectious to Virginia Savoy or Old Dominion have been described (1, 3), Virginia Savoy, Bloomsdale, and the selection of P.I. 179590 were screened for resistance to 7 strains of cucumber virus 1.

Materials and methods.—Plants were grown singly in 2-in. thumb pots. For the temperature studies, plants in the cauline-leaf stage were transplanted to 4-in. pots and held at the various temperatures (Table 1) for 7 days preceding inoculation and throughout the test period.

Strains of cucumber virus 1 (Table 2) were maintained by S. P. Doolittle in plants of *Nicotiana tabacum* L. var. Samsun and *N. glutinosa* L. Inoculum was prepared by grinding infected leaf tissue in a 0.5*M* solution of a phosphate buffer (pH 7) with a mortar and pestle. Inoculations were made with a gauze pad saturated with inoculum and rubbed over the upper surface of the cotyledons (9) or cauline leaves of plants previously dusted with Carborundum.

Greenhouse temperatures, unless otherwise stated, were maintained between 22 and 24°C.

Experimental results.—*Resistance to the spinach blight strain of cucumber virus 1 at 4 temperatures.*—Most plants of the susceptible variety Bloomsdale became infected at 16 and 20°C. and all were infected at 24 and 28°C (Table 1). Severity of the disease in Bloomsdale was directly related to temperature, being

Table 1.—*Numbers of spinach plants infected when a selection of P.I. 179590 and 2 varieties were inoculated with the spinach blight strain of cucumber virus 1 and held at different temperatures (before and after inoculation)*

Temperature (°C)	Number of plants infected[a]		
	P.I. 179590	Virginia Savoy	Bloomsdale
16°	0/28	0/24	14/17
20°	0/27	0/13	16/19
24°	0/36	5/30	30/30
28°	0/38	6/16	26/26

[a] Numerator is number of plants infected; denominator is the number inoculated.

[1] Plant Introduction accession number.

TABLE 2.—*Numbers of spinach plants infected following inoculation of a selection of P.I. 179590 and 2 varieties with various strains of cucumber virus 1*

Virus strains[a]	Number of plants infected[b]		
	P.I. 179590	Virginia Savoy	Bloomsdale
1	0/41	2/24	20/28
4	0/30	11/20	38/38
5	0/30	1/18	23/28
6	40/40	20/20	33/33
7	0/30	8/46	26/26
8	0/39	11/19	29/29
9	0/30	5/18	24/24

[a] The sources of the strains were as follows: 1—type strain obtained from cucumber; 4—originally isolated from cucumber grown in greenhouse in Indiana; 5—originally isolated from cucumber collected from the Chicago Market; 6—originally isolated from *Commelina nudiflora* L. Beltsville, Maryland; 7—originally isolated from spinach grown in Pennsylvania; 8—originally isolated from tomato grown in greenhouse in Ohio; 9—originally isolated from pepper grown in Delaware.

[b] Numerator is the number of plants infected; denominator is the number inoculated.

most severe at 28°. Virginia Savoy resisted infection at 16 and 20°, but some plants became diseased at 24 and 28°C. Reactions of infected plants at these temperatures were similar to those described by Pound and Cheo (7). Inoculated plants of the selection from P.I. 179590 remained healthy at all 4 temperatures.

Further studies at a constant temperature of 32°C indicated that the selection from P.I. 179590 is not immune to the spinach blight strain of cucumber virus 1. At this temperature, 3 of 12 plants became severely diseased in 3 days and were dead in 7 days.

Resistance to isolates of cucumber virus 1.—Fulton (3) found that strains of cucumber virus 1 obtained from infected spinach growing in Arkansas varied in their ability to infect the resistant varieties Virginia Savoy and Old Dominion. Desiccated tissue from plants infected by his most infectious strain was supplied by Fulton, but virus could not be recovered. The strain reported by Doolittle and Zaumeyer (1) to be infectious to Old Dominion was not available. Seven strains (Table 2) from various sections of the United States were obtained from S. P. Doolittle, Beltsville, Maryland. The cotyledons and cauline leaves of plants of the resistant P.I. 179590 and Virginia Savoy and of the susceptible Bloomsdale were mechanically inoculated with these strains.

P.I. 179590 was highly resistant to all strains except strain 6 (Table 2). In 3–4 days, strain 6 (2) induced necrotic spotting and yellowing in inoculated leaves, and severe stunting, yellowing, and necrosis in the young leaves of all inoculated plants. Affected plants were dead in 8–12 days. Virginia Savoy was highly resistant to strains 1, 5, 7, and 9, moderately susceptible to strains 4 and 8, and as susceptible to strain 6 as was P.I. 179590. The symptoms induced in Virginia Savoy by strains 4, 6, and 8 were similar, except that strain 6 induced death of the plants 3–4 days earlier than did strains 4 and 8. Most of the inoculated plants of Bloomsdale became diseased. The reactions of Bloomsdale to any given strain were relatively uniform, but its reactions to the various strains were highly variable. Strains 4, 6, and 8 induced the more severe symptoms, whereas the other strains produced moderate leaf mottling, chlorosis, malformation, plant stunting, and eventual death of some plants.

Inheritance of resistance to the spinach blight strain of cucumber virus 1.—The F_1 and F_2 progenies of crosses between Bloomsdale and the resistant P.I. 179590 and reciprocal were screened for resistance at 24°C in comparison with Virginia Savoy, Bloomsdale, and P.I. 179590 (Table 3). Plants in the F_1 progeny were completely resistant, and the F_2 population segregated approximately 3:1 resistant to susceptible. It appears that resistance to the spinach blight isolate of cucumber virus 1 inherent in P.I. 179590 is controlled by a single dominant gene.

Discussion.—Comparative studies of the resistance of P.I. 179590 and Virginia Savoy to the spinach blight strain of cucumber virus 1 indicate that the resistance inherent in P.I. 179590 is stable at higher temperatures than is that possessed by Virginia Savoy. Also, P.I. 179590 was more resistant to 6 of the 7 strains of cucumber virus 1 than was Virginia Savoy. Isolates obtained from infected plants of Virginia Savoy and Bloomsdale growing in various sections of Maryland were noninfectious to P.I. 179590 under greenhouse conditions. Field trials at Beltsville, Maryland, where spinach blight was prevalent in Virginia Savoy and related hybrids, indicated that the resistance of P.I. 179590 is apparently stable under field conditions.

Plants produced from the original seed lot of P.I. 179590 were relatively uniform in their early-bolting habit but differed widely in plant type and habit of growth. Through plant selection and paired matings, several lines that differ primarily in the degree of leaf savoying have been obtained. Preliminary trials under field conditions indicate that these blight-

TABLE 3.—*Numbers of spinach plants resistant or susceptible to the spinach blight strain of cucumber virus 1 following inoculation of 3 varieties and progenies of crosses between Bloomsdale and the resistant selection of P.I. 179590*

Variety or cross	Generation	Number of plants found as indicated	
		Resistant	Susceptible
Bloomsdale		0	97
Virginia Savoy		52	14
P.I. 179590		80	0
P.I. 179590 × Bloomsdale	F_1	85	0
Bloomsdale × P.I. 179590	F_1	82	0
P.I. 179590 × Bloomsdale	F_2	137[a]	43[a]
Bloomsdale × P.I. 179590	F_2	138[b]	45[b]

[a] Theoretical 3:1 ratio in F_2 would be 135:45.

[b] Theoretical 3:1 ratio in F_2 would be 137:45.

resistant lines, when hybridized with blue-mold-resistant lines, either smooth or savoy types, will produce high yielding, high quality hybrids adapted to areas where these diseases are prevalent.—Plant Industry Station, Beltsville, Maryland, and Winter Garden Substation No. 19, Crystal City, Texas.

LITERATURE CITED

1. Doolittle, S. P., and W. J. Zaumeyer. 1953. A pepper ringspot caused by strains of cucumber mosaic virus from pepper and alfalfa. Phytopathology **43**: 333-337.
2. Doolittle, S. P., and R. E. Webb. 1960. A strain of cucumber virus 1 infectious to blight-resistant spinach. Phytopathology **50**: 7-9.
3. Fulton, J. P. 1950. Studies on strains of cucumber virus 1 from spinach. Phytopathology **40**: 729-736.
4. Jones, H. A., D. M. McLean, and B. A. Perry. 1956. Breeding hybrid spinach resistant to mosaic and downy mildew. Proc. Am. Soc. Hort. Sci. **68**: 304-308.
5. Jones, H. A., B. A. Perry, D. M. McLean, and P. W. Leeper. 1957. From Texas comes Dixie Market. Southern Seedsman **20** (6): 17, 38.
6. Perry, B. A., and H. A. Jones. 1957. Two new spinach hybrids developed for the South. Southern Seedsman **20** (10): 26-27.
7. Pound, G. S., and P.-C. Cheo. 1952. Studies on resistance to cucumber virus 1 in spinach. Phytopathology **42**: 301-306.
8. Smith, L. B. 1920. Breeding mosaic-resistant spinach and notes on malnutrition. Virginia Agricultural Truck Expt. Sta. Bulls. 31 and 32: 137-160.
9. Webb, R. E. 1955. Cotyledonary inoculation, a method for screening spinach for blight resistance. Phytopathology **45**: 635.

PHYSIOLOGY OF FUSARIUM SOLANI F. PHASEOLI IN RELATION TO SAPROPHYTIC SURVIVAL IN SOIL

Otis C. Maloy

Research Forester, Potlatch Forests, Inc., Lewiston, Idaho.

Accepted for publication June 29, 1959.

Portion of a thesis presented in partial fulfillment of the requirements for the Ph.D. degree, Cornell University.

Acknowledgment is extended to Drs. W. H. Burkholder and Martin Alexander for suggestions throughout the study and preparation of the manuscript.

SUMMARY

Two forms of *Fusarium solani* f. *phaseoli* occur both in culture and in nature—a conidial (C) form and a mycelial (M) form. The conversion of the conidial form into the mycelial form is accompanied by a decrease in virulence. These 2 forms were compared in their ability to utilize various root extracts and carbohydrates, to tolerate a number of antibiotics, to colonize several plant tissues saprophytically, and to compete for nutrients in soil. Mycelial production by the M-type was selectively stimulated by root extracts of wheat and alfalfa, and rotations including these plants were shown to result in significant reductions in severity of bean root rot. The M-type produced greater growth on a larger number of the carbohydrates tested and tolerated a greater number of antibiotics and higher antibiotic concentrations than did the C-type. The M-type became predominant in soil under conditions of limited nutrient availability. It is suggested that the M-type will predominate in the prolonged absence of a host plant, which may explain the reduction in disease severity without a reduction of pathogen populations.

The ability of bean root rot fungus, *Fusarium solani* (Mart.) Appel & Wr. f. *phaseoli* (Burk.) Snyd. & Hans., to change in culture and to exist in 2 distinct morphological and physiological forms was recognized by Burkholder (2). When isolated from diseased beans, the fungus produces abundant macroconidia in pseudopionnotes, and the spores in mass are green to blue. Few or no microconidia are produced, and aerial mycelium is lacking. This conidial form (C-type) of the fungus is very pathogenic on beans. After several months in culture, the fungus begins to produce abundant white aerial mycelium, numerous microconidia, and few macroconidia. This mycelial form (M-type) is less pathogenic than the conidial type originally isolated from beans. Burkholder observed that the original virulence was restored to the fungus only after several passages through bean plants. The existence of 2 forms was later observed in a majority of the imperfect fungi studied by Hansen (5), who called the formation of the 2 distinct types (C and M) the "dual phenomenon." Hanson demonstrated that the "dual phenomenon" is a result of heterocaryosis rather than a result of mutation, as suggested by LaRue (6) in the case of *F. solani* f. *phaseoli*.

Estimates of populations of *F. solani* f. *phaseoli* in 39 bean fields revealed no marked differences in the number of infection units between fields with severe root rot and fields with mild root rot (7). Burkholder (2) suggested that the loss of virulence accompanying cultural variation of *F. solani* f. *phaseoli* may explain the decline of root rot severity after several years' absence of the host. This hypothesis was investigated by evaluating the role of the 2 cultural types of *F. solani* f. *phaseoli* in the absence of the host. A comparison was undertaken of the saprophytic abilities of the C- and M-types, *viz.*, which would predominate under saprophytic conditions.

A series of experiments were conducted to compare the 2 fungus types with respect to 3 of the 4 characteristics cited by Garrett (4) as favoring a high saprophytic ability. In addition to comparisons of morphology and pathogenicity, specific investigations involved: 1) the utilization of root extracts by the 2 types, 2) utilization of various carbohydartes, 3) tolerance of antibiotics, 4) saprophytic colonization of plant tissues, and 5) maintenance of type in soil.

Materials and methods.—The 2 types of the pathogen were obtained for study by plating conidia from a virulent isolate of *F. solani* f. *phaseoli* on potato-dextrose agar (PDA containing 1% dextrose). The majority of the colonies that developed were of the C-type, but a few (less than 0.1%) were of the M-type. Transfers of each type were made to slants of PDA. The conidial isolate was maintained by occasional passage through bean and reisolation. A uniform mycelial type was obtained by 2 subsequent dilution platings.

Growth of the 2 fungus types was compared in dilute root extracts and in media supplemented with root extracts. Root extracts were obtained by macerating the roots of 10-week-old plants and filtering. The extracts were sterilized by filtration and diluted 4-fold with sterile distilled water; 25-ml portions dispensed into sterile 125-ml flasks; 0.1 ml of a spore and/or mycelial suspension of either the C- or M-type added; and incubated 6 weeks at room temperature. In a separate experiment, sterile, undiluted root extracts were added to Richards' solution (10) to give a root extract concentration of 5%. Two dextrose concentrations were employed, 0.2% and 1%. In addition, other flasks contained media supplemented with 1 ml of soil extract[1] per 20 ml of medium, and others served as unsupplemented controls. These flasks received fungus suspensions and were incubated 2 weeks at room temperature. Mycelial weights were obtained by filtering the mycelial mats onto 22-mm filter paper disks of uniform weight placed in Gooch crucibles, and drying and weighing the disks.

To determine if one fungus type could utilize a larger number of carbon sources and utilize them more efficiently than the other type, each of 30 carbohydrates was added to Richards' solution to give 400 mg of carbon per 100 ml of medium (equivalent to 1% dextrose). The media were dispensed in 20-ml portions into 50-ml flasks and autoclaved 15 minutes at 15 pounds, 0.1 ml of a spore and/or mycelial suspension of the C- or M-type added, the flasks incubated 12 days at room temperature, and the mycelial weights recorded. Fifteen of the carbon sources were used initially, and the remainder in a second trial with dextrose serving as an internal standard. Because of the viscosity of the medium containing xylan the cultures were centrifuged to remove the mats before they were filtered.

The tolerance of the 2 types of the pathogen to 18 antibiotics (Table 3) was determined since the ability of a fungus to grow in the presence of antibiotics may favor saprophytic survival of that fungus. Filter paper disks were soaked in the antibiotic solutions and placed on plates of PDA seeded with the C- or M-type. The plates were incubated 1 week at room temperature, and the diameters of the inhibition zones recorded, the diameter of the filter paper disk being subtracted.

[1] Prepared by autoclaving 1 kg of soil in 1 liter of tap water and filtering.

The ability of the fungus to colonize nonhost-plant tissues saprophytically, and the influence of a competing microflora on this ability, were determined. Root and stem tissues were obtained from mature field-grown bean, alfalfa, and wheat plants. The tissues were cut into 2-in. pieces (wheat pieces were 4-in. long and included a node) and exposed for 48 hours to an atmosphere of methyl bromide. The tissues were then aerated aseptically for 1 week, and samples plated on PDA to ascertain completeness of sterilization. Nonsterile greenhouse potting soil was placed in screw-top pint jars, and one-half of the jars were autoclaved. Spore suspensions of the C-type were added to one-half of the sterilized and to one-half of the unsterilized jars of soil. The remainder of the jars were infested with the M-type. The spore suspensions were mixed to give 100 spores per g of soil. Twenty-five pieces of each plant tissue were placed in each of 3 replicate jars of soil, and the jars were incubated 6 weeks at room temperature. The plant tissues were removed and washed thoroughly, the epidermal tissues being removed by abrasion. Each piece was then placed in contact with the roots and hypocotyl of a Red Kidney bean seedling growing in steamed soil. Symptoms were apparent, and readings were taken after 2 weeks since a longer time might have resulted in some contamination through water splashing.

Results.—The pathogenicity of individual isolates was determined by mixing the fungus growth on PDA slants with steamed soil, planting with Red Kidney bean, and evaluating the resulting disease. To determine if the severe root rot caused by the C-type was due to numerical advantage over the M-type, cultures of the C-type were washed to remove the conidia and both the spore suspensions and the remaining mycelium were used separately to infest steamed soil. In general, the conidial type produces a very severe root rot accompanied by a marked stunting of the plant, often resulting in death. The mycelial type produces a moderate to severe root rot but no marked stunting. There were no differences between the spores and the mycelium of the C-type in severity of root rot induced.

The growth rates of the 2 fungus types appear to be about the same when grown under similar conditions on PDA. Colony diameters of the C-type were almost identical with those of the M-type.

Mycelial growth of both types on dilute root extracts from the nonhost plants was greater than on Red Kidney bean extracts (Table 1). The M-type, however, did not give greater growth than the C-type on any of the extracts.

The fact that there were no differences in the mycelial weights of either fungus type grown in the unsupplemented control and in any of the supplemented media containing 0.2% dextrose demonstrated that no available carbon was provided by the root extracts. Only the results obtained in the cultures containing 1% dextrose are presented. There were significant responses to the addition of all of the root extracts to the medium containing 1% dextrose

TABLE 1.—*Growth of C- and M-types of Fusarium solani f. phaseoli in diluted root extracts and in a dextrose medium supplemented with root extracts*

Root extract source	Mycelium dry weight (mg) in 2 media			
	Diluted root extracts		Dextrose medium plus 5% root extract	
	C-type	M-type	C-type	M-type
Red Kidney bean (*Phaseolus vulgaris* L.)	5	3	62	56
N-203 bean (*Phaseolus vulgaris* L.)	14	11	62	52[a]
Cabbage (*Brassica oleracea* subsp. *capitata* L.)	5	4	60	57
Corn (*Zea mays* L.)	17	12	63	51[b]
Wheat (*Triticum vulgare* Vill.)	9	6	51	57[c]
Alfalfa (*Medicago sativa* L.)	16	13	60	66[c]
Red clover (*Trifolium pratense* L.)	11	9	49	52
Soil extract			39	39
Control			29	28
LSD 5%	6	1	12	11

[a] Difference between C and M means is significant at the 1% level.
[b] Difference between C and M means is significant at the 5% level.
[c] Difference between C and M means is significant at the 10% level.

(Table 1). The response due to addition of soil extract was not as pronounced as with root extracts, but it did approach significance. On the same medium, in all but 2 instances, the mycelial weight of the C-type was slightly greater than that of the M-type. The 2 exceptions were the higher weights in the mycelial series than in the conidial series on wheat and alfalfa extracts, both differences being significant only at the 10% level. The important consideration is that these are the 2 plants that reduced bean root rot severity in field (wheat) and greenhouse (alfalfa) experiments (7).

A considerable difference was observed between the weights of the C- and M-types in the dextrose medium in the second series of carbohydrates (Table 2), instead of the equal values obtained on dextrose in the first experiment. The second experiment also yielded much lower weights than the first. Since a comparison between the 2 types was desired only on a given medium, the weight of the M-type on dextrose in the second series was raised to equal the C-type on dextrose, and the others raised proportionally. The M-type produced the greater weight in 90% of the comparisons where significant differences occurred. This indicates that the M-type is able to utilize a wider variety of carbon sources more efficiently than the C-type.

An attempt was made to determine the cause of the variation in mycelial weights of the C- and M-types in the second series of carbohydrates. Since the basic medium and the source of inoculum were the same in both series, it was thought that variations in the temperature during incubation might have been responsible. Cultures of the C- and M-types in Richards' solution containing 1% dextrose were placed in incubators maintained at 3, 6, 9, 12, 15, 18, 21, 24, 27,

TABLE 2.—*Relative growth of the C- and M-types of Fusarium solani f. phaseoli in media containing various carbohydrates*

Mycelium dry weight (mg)					
First experiment			Second experiment		
Carbohydrate	C-type	M-type	Carbohydrate	C-type	M-type
Dextrose	61	62	Dextrose	46	46[a]
Fructose	41	44	Raffinose	6	9[b]
Mannose	36	44[b]	Melezitose	41	38
Galactose	16	40[c]	Galacturonic acid	1	3[c]
Rhamnose	15	30[c]	Glycerol	1	2[b]
Sorbose	11	20	Erithritol	18	28[c]
Arabinose	13	61[c]	Dulcitol	9	21[c]
Xylose	41	42	Sorbitol	23	28[b]
Ribose	0	2[c]	Mannitol	34	35
Sucrose	61	64	Inositol	47	53
Maltose	52	56	Adonitol	2	40[c]
Lactose	11	59[c]	Salicin	9	16[c]
Cellobiose	60	62	Dextrin	40	24[c]
Melibiose	0	3[c]	Soluble starch	46	49
Trehalose	56	53	Inulin	5	8[b]
			Xylan	36	28[c]

[a] M-type mycelial weights in the second series were corrected to equal dextrose values.
[b] Difference between C and M means is significant at the 5% level.
[c] Difference between C and M means is significant at the 1% level.

TABLE 3.—*Inhibition of the C- and M-types of Fusarium solani f. phaseoli by various antibiotics*[a]

	Diameter of inhibition zones (mm) resulting from various antibiotics																	
	Aureomycin		Actidione		Terramycin		Pleocidin		Achromycin		Thiolutin		Candicidin		Clavacin		Polymyxin B[b]	
Antibiotic concentration	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M
5000 ppm	19	8[c]	18	43[d]	6	3[c]	9	9	9	11	20	19	16	12[c]	7	10[c]	10	8
2500 ppm	16	0[d]	14	30[d]	4	0[d]	9	8	9	5	16	15	14	12	4	5	6	5
1250 ppm	11	0[d]	12	24[d]	0	0	9	7	7	3[d]	12	10	13	10[c]	2	1	6	3[d]
625 ppm	6	0	11	18[d]	0	0	7	4[c]	6	0[d]	11	9	10	9	0	0	2	0[d]
313 ppm	3	0	9	17[d]	0	0	3	0	0	0	10	8	10	7[c]	0	0	0	0
LSD 5%	8	–	4	13	2	–	4	3	2	4	5	6	3	3	4	6	4	4
LSD 1%	11	–	5	17	3	–	5	4	3	5	7	8	5	5	5	8	5	5

[a] No inhibition at highest concentration of streptomycin, penicillin, chloromycetin, neomycin, streptothricin, tetracycline, amphomycin ,virideogrisein, or tyrothricin.
[b] Highest concentration 3000 ppm.
[c] Difference between C- and M-type is signicant at the 5% level of probability.
[d] Difference between C- and M-type is significant at the 1% level of probability.

30, or 35°C and incubated 2 weeks, after which the mycelial weights were obtained. The mycelial weights of the 2 types were equal only within a range of 6 degrees (21–27°). It seems possible that a change in mean temperature of several degrees for a week or so might have resulted in the difference observed in the 2 series.

Of the 18 antibiotics tested, aureomycin, actidione, terramycin, pleocidin, achromycin, thiolutin, candicidin, clavacin, and polymyxin B inhibited growth of the bean root rot fungus. The M-type was more tolerant of 7 of these 9 antibiotics than was the C-type, and inhibition was not apparent except at concentrations higher than that inhibitory to the C-type (Table 3).

There was little difference in the colonization of the different plant tissues by either fungus type in sterile soil (Table 4). In unsterile soil, however, the ability to colonize these tissues was reduced significantly, particularly on wheat tissues. It also appears that the M-type is better able to colonize certain nonhost tissues (e.g., alfalfa) than other tissues in the presence of a competing microflora. It should be realized that the assay was one based on a pathogenic reaction, and may have obscured any truly saprophytic abilities that the M-type may have since the M-type is the weaker pathogen of the 2 fungus types.

To determine if one fungus type would predominate over the other under conditions of limited nutrient supply, a mixture of conidia of the C- and M-types was used to infest soil amended with 1% soybean meal and unamended soil at the rate of 1000 spores per g of soil. Samples of infested soil were plated periodically and the numbers of C- and M-type colonies recorded. There was a continuous increase in numbers of the fungus (the total of both types) up to 15 weeks in the unamended soil, with a slight decline at 20 weeks (Table 5). The increase was much more pronounced and the subsequent decline more noticeable in the amended soil. There was approximately an equal proportion of each fungus type at the beginning of the experiment, and after incubation for 1 week the percentage of M-type dropped to about 15% in both treatments. This increase in the relative proportion of C-type is easily explained since a conidial type will have the initial advantage in producing propagative units. It is interesting, however, that a large number of these C-type propagules must have developed into the M-type, since the recovery of the original balance occurred rather rapidly. The recovery occurred about 5 weeks earlier in the unamended soil than in the amended soil. M-type predominance did not occur in amended soil within the duration of the experiment and may have been due to the availability of a readily assimilated substrate, which would result in continued conidial production. Fifteen weeks after the beginning of the experiment the predominance of the M-type was significantly greater than at the beginning. In the amended soil there appeared to be a stabilization of types after 6 weeks, but this might have been altered had the experiment been continued for an extended period. On the basis of these results, however, it seems that the M-type tends to predominate under limited nutritional conditions. Differences between the proportion of M-type in amended and

TABLE 4.—*Saprophytic colonization of root and stem tissues of host and nonhost plants by the C- and M-types of Fusarium solani f. phaseoli in the presence and absence of a competing microflora*

	% of plant tissue colonized			
	C-type		M-type	
Plant tissue	Sterile soil	Unsterile soil	Sterile soil	Unsterile soil
Red Kidney bean	97	53[a]	93	19[a]
Alfalfa	100	54[a]	84	50[a]
Wheat	81	13[a]	71	2[a]
LSD 5%	15	31	16	31
LSD 1%	21	45	24	45

[a] Difference between means is significant at the 1% level.

unamended soils were significant in 4 of the last 5 sampling periods (Table 5).

The influence of rotation crops on maintenance of the fungus type in soil was studied by isolating *F. solani* f. *phaseoli* from bean plants grown in greenhouse soil previously cropped to various rotation plants. These isolates were maintained on PDA and examined at 30-day intervals to determine the cultural type. On isolation, all of the cultures were the C-type. The tendency of an isolate to convert to the M-type was associated with low pathogenicity. The isolates obtained from beans grown following wheat ranked lowest in pathogenicity and were the isolates that most readily developed into the M-type. The relationship between pathogenicity and complete mycelial conversions is particularly strong, since 33% of the 3 least pathogenic groups (red clover, alfalfa, and wheat) were completely mycelial after 5 months in culture, as compared with 11% of the 3 most pathogenic groups. An unexplained reversion from M- to C-type took place in 1 of the isolates obtained from bean plants grown in manure-amended soil. When transfers were made, an attempt was made to transfer both spores and mycelium if possible, and this reversion of type may have been due to heterocaryosis.

Discussion.—The possible importance of the M-type of *F. solani* f. *phaseoli* in saprophytic persistence of this fungus in soil was studied by applying the criteria for saprophytic ability suggested by Garrett. Although the complete ecological importance of these 4 criteria is not known, it appears that they offer, in part, a means of comparing the competitive saprophytic abilities of 2 or more organisms. The importance of antibiotic production in favoring saprophytic survival has not been demonstrated, although Brian (1) discussed this subject in some detail and considers antibiotic production to have some ecological significance. Production of antibiotics was omitted from this study because of the extensive and complex techniques that would have to be employed to make an adequate survey for antibiotic production. It should be recognized, however, that there are a large number of other factors, physical, chemical, and biological, that affect the ability of an organism to compete successfully as a soil saprophyte.

The fact that growth of both types of the fungus occurred on root extracts from a number of nonhost plants, and in some instances the growth on these exceeded that on root extracts from a suitable host plant, is strong evidence against a starvation hypothesis in explaining the effect of crop rotation in reducing bean root rot severity. Furthermore, the root extracts that selectively stimulated the less pathogenic M-type were from plants that reduced root rot severity in field and greenhouse experiments. Extracts from wheat and alfalfa roots stimulated the growth of the M-type to a greater extent than the C-type, whereas the reverse occurred with the other root extracts employed.

The M-type proved able to give significantly greater growth than the C-type on the majority of the carbohydrates tested, which may indicate that the M-type is better able to adapt to different nutritional conditions than the C-type and is thereby more likely to compete successfully for an available substrate.

The M-type was more tolerant of a greater number and higher concentrations of antibiotics than was the C-type. The ability to tolerate a comparatively large number of antibiotics at concentrations higher than would be expected under natural conditions is a factor that would favor saprophytic survival on, and active competition for, available substrates in the soil. Butler (3) showed that the higher saprophytic ability of *Curvularia ramosa* (Bainier) Boed. than that of *Helminthosporium sativum* Pam., King & Bakke was associated with the ability of the former organism to tolerate a larger number of antibiotics than the latter fungus. Park (8, 9), however, said that tolerance to antagonistic bacteria and fungi has little or no relation to the saprophytic survival of fungi in the soil.

Saprophytic colonization of nonhost-plant tissues is a factor that would enhance the survival of a pathogen in the absence of a suitable host plant. The bean root rot pathogen was shown to be able to colonize both leguminous and nonleguminous nonhost-plant tissues. Saprophytic survival is favored considerably if the organism can compete successfully with the normal soil microflora in colonizing these nonhost-

TABLE 5.—*Population trends and frequency of C- and M-types of Fusarium solani f. phaseoli in sterilized soil either unamended or amended with 1% soybean meal*

	Total number of propagules and percentage of M-type at various times							
	0	1 week	1½ weeks	6 weeks	10 weeks	15 weeks	20 weeks	LSD 5%
Numbers of propagules								
UA[a]	1000[b]	886	4316	6400	9200	15,100	11,520	
A[c]	1000	1,500,000	2,532,000	435,000	660,000	280,000	13,520	
% M-Type								
UA	54	16	42	57	59	71	70	14
A	54	13	11[d]	43	42[d]	38[d]	38[d]	23

[a] Unamended soil.
[b] Calculated from the number of spores per ml of suspension used to infest the soil and expressed as the number per g of soil.
[c] Soil amended with 1% soybean meal.
[d] Difference between unamended and amended soil is significant at the 1% level of probability.

plant tissues. Though no marked differences were observed in the colonization of various plant tissues by the C-type in the absence of a competing microflora, there were significant differences when a normal soil microflora was present. The marked reduction in percentage of effective colonization in the case of wheat tissues may partially account for the reduction in root rot severity in beans that follow wheat.

The ability of the fungus to increase on a minimal substrate such as soil would favor survival of the fungus for long periods in the absence of a suitable host plant. The tendency for the M-type to predominate would help explain the lessening of root rot severity although no reduction in total numbers of the fungus has occurred. It is noteworthy that, of the cultures of *F. solani* f. *phaseoli* isolated from beans growing in soil previously cropped with nonhost plants or amended with organic materials, the isolates that were lowest in pathogenicity were the ones that 1) tended to become mycelial and 2) were isolated from beans grown following crops that had been shown to reduce root rot severity.

Although most of the evidence suggested that the M-type is the better saprophyte of the 2 forms, and therefore is better able to persist in prolonged absence of the host, there were 2 factors indicating that the M-type is not as efficient in saprophytic ability as the C-type: 1) the M-type produced a maximum amount of mycelium over a narrower temperature range than did the C-type; 2) in the presence of a competing microflora the M-type did not colonize certain plant tissues to as high a degree as did the C-type. A possible explanation relating to the relative pathogenicity of the 2 types has been offered for the latter situation.

It is possible that the "dual phenomenon" enhances the ability of *F. solani* f. *phaseoli* to survive in the soil and may explain why reduction in disease severity occurs in the absence of a corresponding reduction in pathogen populations. The evidence further indicates that certain root extracts selectively favor predominance of one fungus type over the other and may account for the beneficial effects of certain rotation crops in reducing disease severity.—Department of Plant Pathology, Cornell University, Ithaca, New York.

LITERATURE CITED

1. Brian, P. W. 1957. The ecological significance of antibiotic production. *In* Microbial Ecology. Seventh Symp. Soc. Gen. Microbiol., p. 169-188.
2. Burkholder, W. H. 1925. Variation in a member of the genus Fusarium grown in culture for a period of five years. Am. J. Botany **12**: 245-253.
3. Butler, F. C. 1953. Saprophytic behavior of some cereal root rot fungi. II. Factors influencing saprophytic colonization of wheat straw. Ann. Appl. Biol. **40**: 298-304.
4. Garrett, S. D. 1956. Biology of Root Infecting Fungi. Cambridge Univ. Press, New York, 293 p.
5. Hansen, H. N. 1938. The dual phenomenon in imperfect fungi. Mycologia **30**: 442-455.
6. LaRue, C. D. 1925. Loss of virulence in fungi. Science **62**: 205-206.
7. Maloy, O. C., and W. H. Burkholder. 1959. Some effects of crop rotation on the Fusarium root rot of bean. Phytopathology. **49**: 583-587.
8. Park, D. 1957. Behavior of soil fungi in the presence of bacterial antagonists. Brit. Mycol. Soc. Trans. **40**: 283-291.
9. Park, D. 1957. Behavior of soil fungi in the presence of fungal antagonists. Brit. Mycol. Soc. Trans. **40**: 358-374.
10. Riker, A. J., and Regina S. Riker. 1936. Introduction to research on plant diseases. 117 p. Published by the Authors, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

THE EFFECT OF ENVIRONMENT ON GERMINATION OF SPORIDIA IN CRONARTIUM RIBICOLA

Robert V. Bega

Plant Pathologist, Pacific Southwest Forest and Range Experiment Station, Forest Service, U. S. D. A., and Associate in Plant Pathology, University of California, Berkeley.

Accepted for publication July 3, 1959.

The author expresses his appreciation to Drs. W. C. Snyder, H. N. Hansen, and J. W. Kimmey for helpful criticism.

SUMMARY

Sporidia of *Cronartium ribicola* were subjected to various environmental conditions to determine the effect of environment on germination. Type, time, and vigor of germination were given primary emphasis because germination *per se* was found not to be an accurate measure of sporidial response to environment.

Artificial light vs. dark was studied as to its effect on type and vigor of sporidial germination. No significant effect of light was noted.

In laboratory studies, primary sporidia were capable of producing up to six successive generations of sporidia, but the fourth to sixth generations appeared to be of minor importance in disease spread. Secondary and tertiary sporidia, however, were produced in great enough abundance to warrant consideration. Indirect germination, therefore, is a means of vegetative perpetuation and can be an aid in dispersal and longevity of sporidia. A sporidium, with its ability to germinate either directly or indirectly, can act as an organ of infection or as an organ of sporulation.

Germination of primary sporidia occurred over a range of pH from 3.0 to 11.0. The optimum pH was 3.0 for direct germination and 7.0–9.0 for indirect. It was inhibited at pH 2.0. The optimum pH for vigor of germination (length of germ tube) was at 4.0 and decreased with increase in pH. Secondary sporidia showed the same reactions.

Germination occurred from 0.5–1° to 24°C at pH 6.8 and from 0.5–1° to 28° at pH 4.0. Time was found to be an important factor in vigor of germi-

nation. At the higher temperatures sporidia exhibited greater vigor at pH 4.0 after 6 hours than at pH 6.8 after 24 hours. The influence of time-temperature-pH on direct vs. indirect germination was studied. Direct germination was favored at all temperatures studied at pH 4.0.

Sporidia germinated on needles of *Pinus lambertiana* from 0.5–1° to 28°C. Vigor of germination was exemplified by the predominance of 2–3 germ tubes per spore and by branching of germ tubes. Germ tube length increased as the temperature increased to 16–20°C after which length decreased to 4.2 μ at 28°C. Few secondary sporidia were formed on the needles at any of the temperatures studied. On needles of conifer species other than white pines indirect germination increased, and direct germination consisted predominantly of one germ tube per spore. No significant difference in vigor of germination was noted on needles of 6 species of white pines tested.

Introduction and review of literature.—The disease "white pine blister rust" is caused by the heteroecious rust fungus *Cronartium ribicola* Fischer, which produces 5 different spore stages during its life cycle. The infection chain is facultatively heterogeneous. Infection of white pines is established only by the airborne sporidia. The ability of this spore stage to cause infection and thereby complete the life cycle depends largely on favorable environmental conditions between production on ribes and penetration of pine needles. The primary limiting factors for infection have been generally defined as temperature and moisture. Since the sporidium is a somewhat delicate, thin-walled organ, a third factor, time, is also important.

The distance of spread of sporidia from ribes to white pines (based on pine infection data) is normally short; 900 feet to 1 mile is considered to be a safe distance between the two hosts for practical control. Infections do occur, however, over much greater distances from any known ribes host. One purpose of this study was to determine the means by which sporidia can survive longer under various conditions and possibly cause infection at greater distances from their source than previously reported. It was found that certain environmental factors, notably kind and pH of substrate on which the spores germinate, have an effect on the type, time, and vigor of germination. These factors have not previously been reported as affecting this spore stage.

The term "direct germination" as used herein means simply the production of germ tubes; "indirect germination" refers to the formation of secondary, tertiary, etc., sporidia.

The influence of temperature on germination of primary sporidia was studied by Hirt (8), who used telia from naturally infected *R. nigrum* as a spore source. He found that they germinated at temperatures between 0–1°C and 21°C, inclusive. The shortest time necessary for germination was about 4 hours at 10–12°, 7 hours at 12–14°, and 5 hours at 17–18°C. York and Snell (15) found sporidia capable of germinating 1 hour after they were cast.

Although the production of secondary sporidia is common in several genera of the Uredinales, few studies have been conducted on their physiology. De Bary (5), writing in general on the Uredineae, said: "It is moreover not unusual for the germ-tubes of sporidia, when they cannot penetrate at once into the host, first to abscise a secondary sporidium at their apex, which has the characters of the original sporidium." Spaulding and Rathbun-Gravatt (12) reviewed the literature up to 1926 on the occurrence of secondary sporidia in the rusts in general. Sappin-Trouffy (10) first reported their production on the genus *Cronartium flaccidum* Alb. & Schw. In 1919 Colley (4) was first to report their occurrence in *C. ribicola*. Clinton and McCormick (3) also reported them in this species and suggested that formation of a secondary sporidium may tide this spore stage over unfavorable conditions of infection. Spaulding and Rathbun-Gravatt (11, 12) found secondary sporidia common in their investigations and regarded the occurrence as "vigorous sporidial germination." They concluded that both fresh and stored telia produce primary sporidia, which in turn produce secondaries. They suggest that icing slides of primary sporidia stimulated the production of secondaries, whereas preliminary drying had little effect. Some slides that were alternately wetted and dried produced secondary sporidia. They briefly mentioned the occurrence of tertiary sporidia.

Hirt (8) described and illustrated secondary sporidium formation on water agar. He stated that tertiary sporidia are uncommon, usually occurring only at the maximum temperature permitting sporidium germination. He further reported that, under both natural and controlled conditions, primary sporidia germinate both by germ tube and by secondary sporidia. At temperatures approaching freezing, very few produced secondary sporidia, but at 10–18°C they were abundant and germinated immediately, forming true hyphae which elongated rapidly. He found that sporidia at 21°germinate by short, stout germ tubes and rarely by an undeveloped, minute secondary sporidium. He considered the germ tubes to be abnormal, with little possibility of infecting pine. Reed and Crabill (9), working with *Gymnosporangium juniperi-virginianae* Schw., found that secondary sporidia were produced abundantly only if the primaries were kept moist constantly from the time of production until germination.

Materials and methods.—Aeciospores, collected from sugar pine (*Pinus lambertiana* Dougl.) in northern California, were used as original inoculum in these studies. Because of the chance of contamination by *Cronartium occidentale* Hedgc., Bethel & Hunt, urediospores from plants growing in the wild were not used. All infected ribes used were guarded against contamination by urediospores and aeciospores of this latter fungus by keeping the inoculated plants in closed chambers of the greenhouse or laboratory.

Urediospore inoculum was maintained on potted ribes in greenhouse cubicles at about 25°C. *Ribes*

aureum Pursh, *R. nevadense* Kell., and *R. roezli* Regel were employed.

Except where noted, the experiments were conducted in the climate-control mycotron of the Department of Plant Pathology, University of California, Berkeley.

Teliospores were obtained from the same three *Ribes* spp. by keeping infected plants at a constant temperature of 20°C in special greenhouse cubicles, or at 16°C in the climate-control chambers. Preliminary experiments showed no difference in sporidium germination between telia formed at these two temperatures or among telia formed on different host plants.

At the outset, several problems arose as to the age and type of sporidia obtained by swirling germinating telial columns in a drop of water or by removing the columns from an agar surface after they had cast their sporidia. In addition, to study the effect of various factors on secondary sporidia, it was desirable to have them of the same age and free of surrounding primary sporidia, which led to confusion in counting spores or measuring germ tubes. To overcome these problems the "plate replacement technique" was designed.

In this technique, telia were attached to a 2-mm³ or larger block of water agar placed on the inside of a Petri-dish lid; the lid was then replaced and the sporidia were allowed to shoot onto an agar surface (Fig. 1). Crest of production was reached in 12–15 hours (Bega, 2), at which time the bottom half of the dish was removed and replaced by one containing the desired substrate. After sporidia had been allowed to cast onto the new substrate for 1 hour (or less, depending on the number of telia used and the number of spores desired), the telia were removed and the sporidia of known age were then subjected to whatever test was being conducted. If secondary sporidia were being studied, the same technique was employed, and after 12–15 hours a block containing the cast primary sporidia was cut out of the agar and placed on the under side of another lid. Secondary sporidia were then allowed to be cast onto whatever substrate was placed in the bottom of the dish. The same procedure was used for studying tertiary, quaternary, etc., sporidia. In some instances it was not necessary to replace the bottom half of the dish, e.g., in the pH studies presented in this paper. In this instance, after the telia had reached their germination crest, the lid of the dish was merely rotated a few degrees and sporidia were allowed to cast onto a new section of the substrate for the desired period, after which the block containing the telia was removed.

Sporidia were subjected to various temperatures, substrates, or lengths of time. At least 100–200 sporidia were counted at 100× and 440× for each treatment; in many cases 500–1000 were counted. The number that had germinated directly, indirectly, or not at all was recorded, as well as the average length of germ tubes. When germination tests were conducted for 24 hours or more, primary sporidia often had produced secondaries, which then proceeded to germinate either directly or again indirectly, making spore counts difficult. To overcome this difficulty the spores were stained with a drop of 0.1% cotton blue in lactophenol, which made the empty sporidia stand out clearly.

Several experiments were conducted under controlled conditions to determine what factor or factors had the greatest effect on type, time, and vigor of germination. (The length of germ tubes formed during direct germination is used as a measure of vigor of germination.) In many tests a few spores produced germ tubes up to 140 μ long 24 hours after germination. Because these were rarities they were not considered in figuring the average length. In the tables, per cent of germination is rounded off to the nearest whole number.

To study the effect of environmental factors on germination of sporidia on pine needles, the needles were cut into 20-mm lengths and fixed to 22-mm-square glass cover slips that were coated with a thin film of lanolin. The cover slips were then placed on a layer of 1.5% water agar in Petri dishes. Usually 5–10 needle pieces were used per cover slip. Sporidia were cast onto them by the plate replacement method and, after being subjected to the various conditions, were stained with 0.1% cotton blue in lacto-phenol and examined with an Ultrapak microscope. The stain was sprayed on with an atomizer so that ungerminated sporidia would not be washed off. Entire needles were used in some tests.

Experimental results.—The fact that sporidia of *C. ribicola* germinate either by production of germ tubes or by formation of secondary sporidia was obvious in preliminary tests.

Indirect germination takes place by the formation of a pointed projection from the mother spore, which for all intents and purposes resembles a sterigma. On the tip of this projection a new spore is formed. In the process the cytoplasm moves into the new cell, leaving the mother spore and its sterigma completely empty. This process is true of germination from primary to secondary, secondary to tertiary, tertiary to quaternary, etc. Vegetative perpetuation of sporidia has been carried through six generations by the plate replacement method. (The term "generation" as used here denotes the formation of a spore from a primary sporidium, one from a secondary sporidium, etc.) With this method, however, sporidia of the fourth to sixth generations rarely detached themselves from the preceding spore. It was not at all uncommon to find sporidia of these latter generations attached to empty

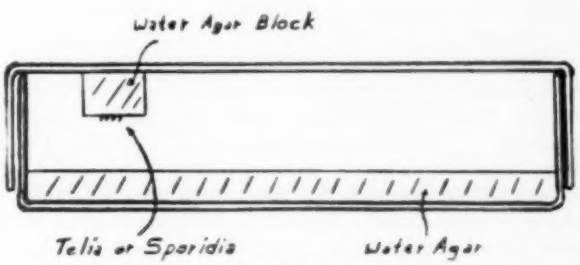


Fig. 1. Diagram of a Petri dish as used in the plate replacement method.

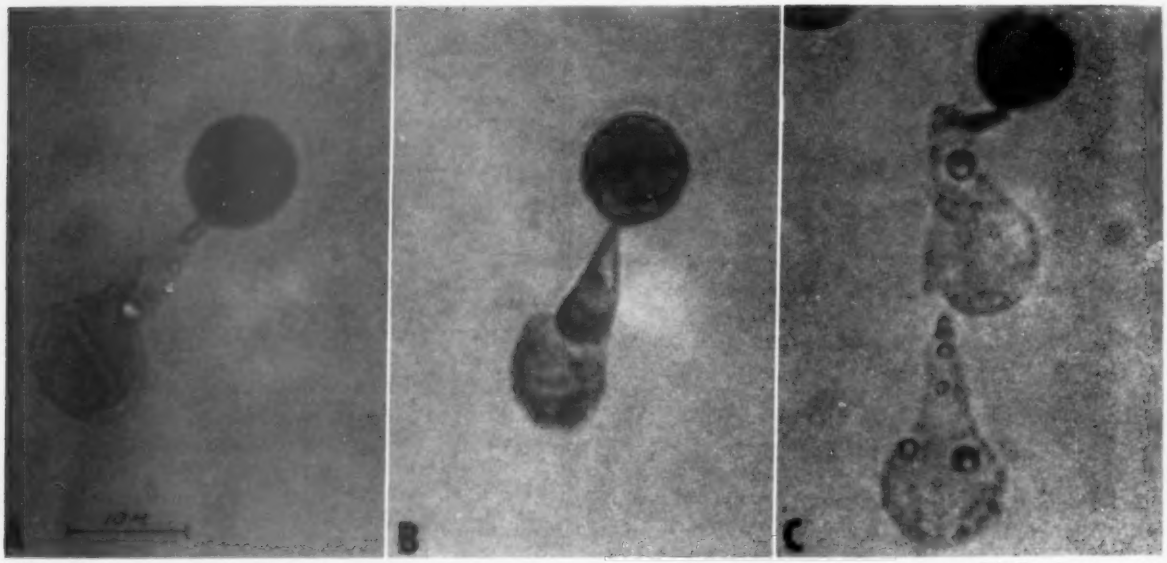


Fig. 2. Indirect germination of sporidia. **A)** Production of a secondary sporidium by a primary. **B)** Production of a tertiary sporidium by a secondary. **C)** Production of a quaternary sporidium by a tertiary with the empty secondary and tertiary spore sacks still attached.

spore sacks. Normal direct germination was observed in all spore generations.

It is generally assumed that, in the formation of spore stages, all nutrients necessary for vegetative perpetuation are obtained from the preceding sporidium. For this reason each spore generation should be successively smaller than its preceding stage. In the present studies spores appeared to become smaller, especially from the fourth to sixth generation, but they were no less perfect, as was evidenced by their normal germination. Fig. 2 illustrates the production of various generations and shows that the process is the same regardless of the number of generations. Size differences of sporidia in this illustration are due primarily to differences in age at the time of photographing.

Light.—The effect of light vs. dark on type and vigor of germination has not been studied with sporidia of *C. ribicola*. Hirt (8) and Spaulding and Rathbun-Gravatt (12) studied the effect of sunlight on germination, but their experiments were designed to test the viability of sporidia after exposure to varying periods of sunlight.

In the present studies, sporidia to be tested were obtained by the plate replacement method on 1.5% water agar at pH 6.6 (unadjusted). Germination was carried out for 24 hours in a 15°C mycotron chamber with continuous 770 ft-c of light. The plates exposed to continuous light were merely placed in the chamber so that they were directly under the lights. Those exposed to the dark were wrapped in four layers of light-proof black cloth over which was placed one layer of white cheesecloth. The sporidia germinated in the dark were never exposed to light. This was accomplished by wrapping the plates in the black cloth as soon as the telia were placed inside. After 15 hours the lid of the dish was rotated a few predetermined degrees to cast sporidia on a new section of the agar without removing the cloth. (The cloth was wrapped loosely enough to permit easy rotation of the lid of the dish.) After allowing the sporidia to cast onto this new section for 2 hours, the lid was again rotated and the experiment considered begun.

The results of three experiments (Table 1) indicate very little difference in sporidial germination response in the light or in the dark.

pH.—According to Gottlieb (7) most fungus spores germinate over a wide range of pH but tend to be acidophilic. Only rarely is germination favored by an alkaline reaction. Webb (13, 14) studied the effect of pH on spore germination of several species of fungi and found that, in general, the majority showed maximum germination at relatively high acidity, namely, pH 3.1. At pH 2.8 and lower, inhibition of germination was evidenced.

In the present studies the effect of pH, from 2.0 to 11.0, on sporidium germination was investigated with 2% water agar as the substrate. The pH of the water agar was adjusted to various concentrations with 0.1*N* and 1*N* HCl and 1*N* NaOH. Measurements were made with a glass electrode pH meter.

Six individual factors were studied: pH, time, total

TABLE 1.—*The effect of light vs. dark on sporidial germination at 15° C*

	Light				Dark			
Trial	% Germ.	% D[a]	Av. LGT[b]	% I[c]	% Germ.	% D[a]	Av. LGT[b]	% I[c]
1	87	30	56.0	70	91	27	75.3	73
2	93	13	82.3	87	97	20	73.1	80
3	97	28	98.7	72	98	23	96.3	77

[a] Direct germination.
[b] Average length of germ tubes (in μ) during direct germination.
[c] Indirect germination.

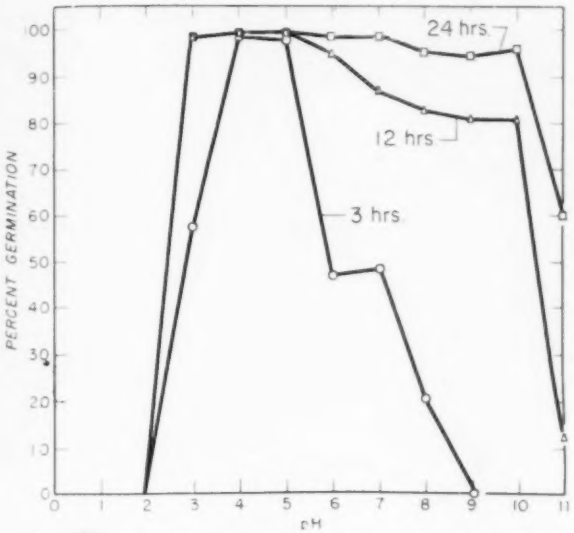


Fig. 3. Effect of pH on germination of sporidia after 3, 12, and 24 hours at 15°C.

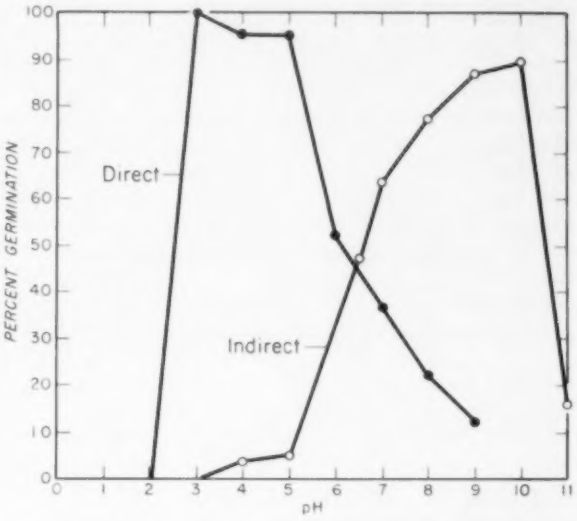


Fig. 4. Effect of pH on direct and indirect germination of sporidia at 15°C. Germination time 24 hours.

germination, direct germination, length of germ tube, and indirect germination. The plate replacement method was used to secure sporidia of equal age. All plates were checked for pregermination of sporidia before the tests were considered begun. Germination was at 15°C.

Germination *per se* occurred over a wide range from pH 3.0 to 11.0. Percentage of total germination after 24 hours dropped from 99% at pH 3.0 to only 60% at pH 11.0. Superficially this would appear to mean that sporidia germinate equally well over this entire range. Judging by vigor of germination, however, they do not. Fig. 3 shows the effect of pH on total germination after three periods: 3, 12, and 24 hours.

The effect of pH on type of germination is shown in Fig. 4. Direct germination was favored by acidity, both the optimum and the maximum occurring at pH 3.0. Indirect germination was favored by an alkaline substrate, with an optimum at pH 9.0–10.0 and a sharp decline at 11.0. Production of secondary sporidia occurred, however, at pH 4.0 to 11.0. No data are presented in Fig. 4 for direct germination at pH 10.0 and 11.0, because at these two units those spores that did not produce secondary sporidia formed thick, blunt germ tubes (24×4.2 μ) and could not be considered normal direct germination. It should also be pointed out that the data presented on direct and indirect germination are taken from the graph illustrating total germination.

Vigor of germination at different pH units could not be presented completely without also including the factor of time. Fig. 5 illustrates the pH effect on length of germ tubes of primary sporidia at 3 different time intervals. Here the optimum was attained at pH 4.0 after 12 and 24 hours (very strikingly at 12 hours) and at pH 5.0 after only 3 hours. At pH 10.0 and 11.0 the germ tubes appeared malformed, being 24×4.2 μ.

The effect of pH on germination of secondary sporidia was also studied. These tests were run for only 12 hours. The same trend was noted for the individual factors as was found with primary sporidia. Total germination ranged from pH 3.0 to 11.0. Direct germination ranged from 3.0 to 7.0, with the optimum at 4.0–5.0. Indirect germination ranged from 4.0 to 11.0, with an optimum of 90–95% producing tertiary sporidia at pH 7.0–9.0. The optimum for germ tube length was at pH 4.0, with a germ tube average of 70 μ.

Time-temperature-pH.—In the present investigations the effect of temperature on sporidial germination was studied on 2% water agar. Because of what had been learned about pH effects, tests were run at two units of pH, 4.0 and 6.8. The pH of 4.0 was obtained with 0.1*N* HCl; pH 6.8 was unadjusted water agar. Electrically heated incubators, which maintained an accuracy of ±0.5°, were used at the following tempera-

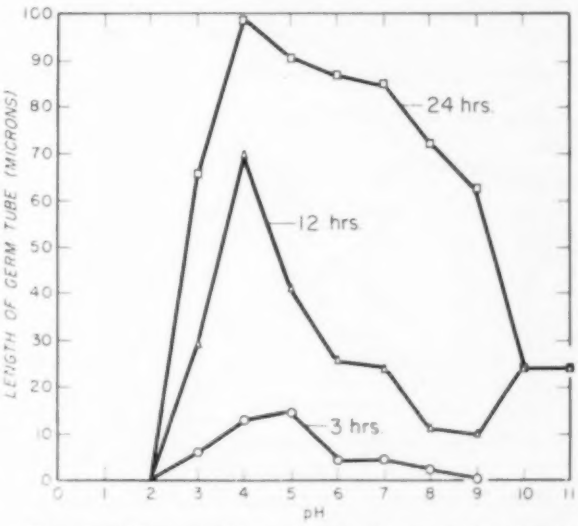


Fig. 5. Effect of pH on length of germ tube after 3, 12, and 24 hours at 15°C.

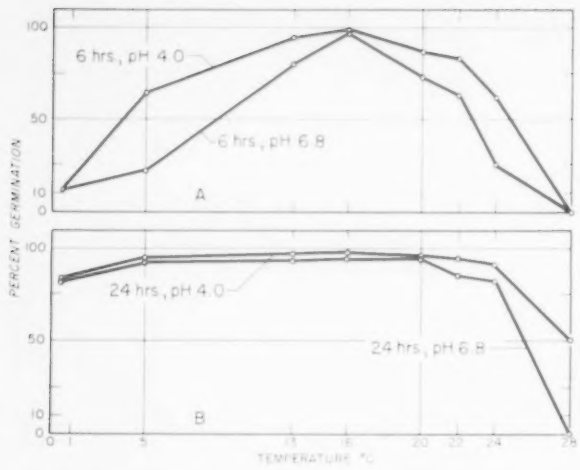


Fig. 6. The effect of time, temperature, and pH on germination of sporidia. **A)** 6 hours. **B)** 24 hours.

tures: 0.5–1°, 5°, 13°, 16°, 20°, 22°, 24°, and 28°C. Temperature, pH, time, total germination, direct germination, length of germ tube, and indirect germination were studied. The plate replacement method was used to ensure sporidia of equal age.

At pH 6.8 sporidia were found to germinate from 0.5–1° to 24°C after both 6 and 24 hours. The optimum was at 16°C, with 95% germination after 6 hours, but after 24 hours the optimum was a range of 5–20°, with germination of 94–96% (Fig. 6). At pH 4.0, sporidia germinated 0.5–1° to 24° after 6 hours and at 0.5–1° to 28° after 24 hours. The optimum was at 16°C, with 98% germination after 6 hours while after 24 hours the optimum range was from 5° to 24° with 93–94% germination.

Direct germination was favored at pH 4.0 at all temperatures studied. An increase in direct germination was noted as temperature increased, except at 16°, where there was a drop to 69%, and then a steady rise from 20° to 24°. At pH 6.8, direct germination was high at 5°, dropped at 16°, and rose again at 20°.

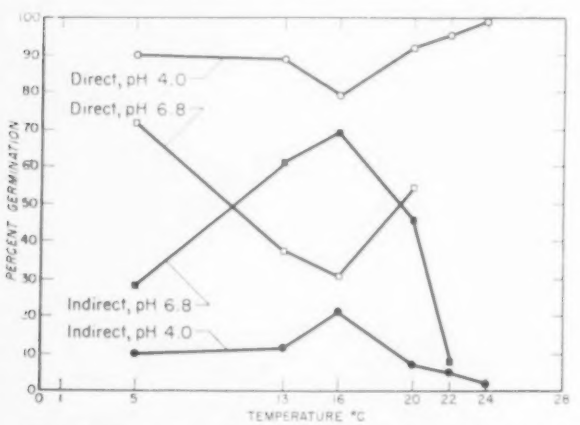


Fig. 7. The effect of temperature and pH on direct and indirect sporidial germination. Germination time 24 hours. No record is presented at the minimum and maximum temperatures, because of the small size of the germ tubes.

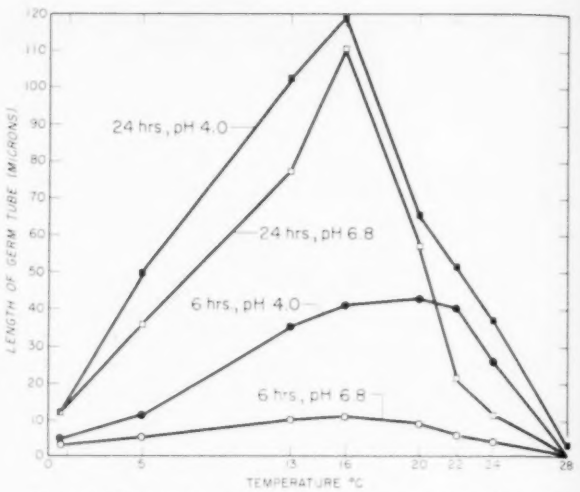


Fig. 8. Effect of time, temperature, and pH on the vigor of germination of sporidia.

The results after 24 hours' germination are presented in Fig. 7. No record is presented for certain temperatures, because the germ tubes were too short to ascertain correctly whether the germination was direct or indirect.

Fig. 8 shows vigor of germination at different temperatures after 6 and 24 hours at pH 4.0 and 6.8. At both pH units optimum vigor was displayed at 16° after 24 hours, but after only 6 hours the optimum at pH 4.0 was found to be 20°C. Very little increase in germ tube length is noted during the time interval between 6 and 24 hours at the temperatures above 20°. It is also of interest to note that sporidia showed greater vigor after 6 hours at pH 4.0 than after 24 hours at pH 6.8 at the higher temperatures. At 28° germ tube length averaged only 3.2 μ.

Pine needles.—Germination of sporidia cast directly onto the surface of needles was studied with respect to species of conifer as well as to type and vigor of germination. After sporidia were obtained by the plate replacement method, the needles were sprayed with sterile distilled water and subjected to the conditions

TABLE 2.—*Germination of primary sporidia on needles of Pinus lambertiana at various temperatures after 24 hours*

Temp. (°C)	% Germ.	% D [a]	Av. LGT [b]	% I [c]
0.5–1	55	([d])	10.6	([d])
5	91	100	30.4	0
13	96	99	74.1	1
16	95	99	82.3	1
20	95	99	77.1	1
22	89	100	34.6	0
24	80	100	19.8	0
28	2	([d])	4.2	([d])

[a] Direct germination.
[b] Average length of germ tubes (in μ) during direct germination. Only the longest germ tube per spore was measured.
[c] Indirect germination.
[d] Not determined, because of small size of germ tube.

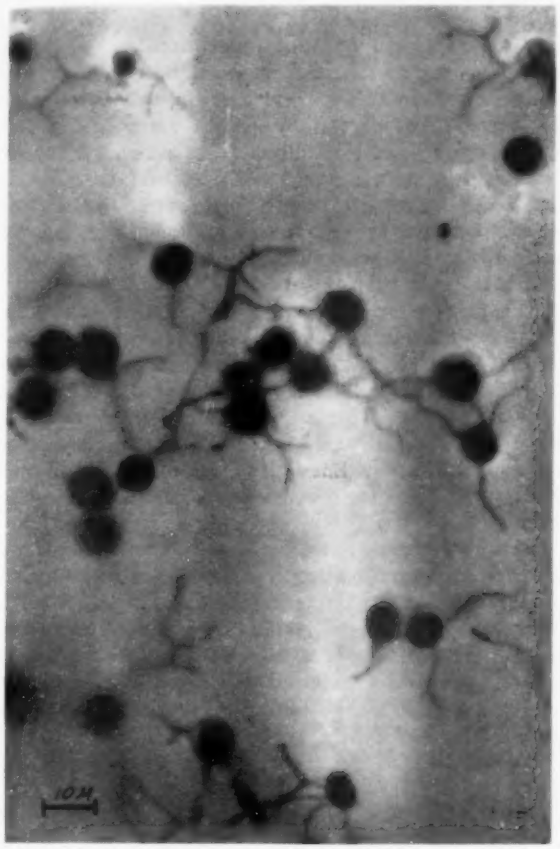


Fig. 9. Germination of primary sporidia on needles of *P. lambertiana* at 16°C. Note predominance of 2–3 germ tubes per spore and branching of germ tubes.

being studied. In the experiments conducted at different temperatures, however, it was found that a film of moisture could not always be maintained on the needles at temperatures of 16–22°C. Therefore, entire needles were laid on the agar without using coverslips, and then a film of water could be maintained.

In the temperature tests, needles of *Pinus lambertiana* were used at various temperatures (Table 2). All tests were conducted in the dark because darkness was found in preliminary experiments not to be a limiting factor. Sporidia were allowed to germinate 24 hours before being sprayed with cotton blue stain.

As found in the experiments on water agar, sporidia germinated at temperatures from 0.5–1° to 28°C. No tests were conducted above 28°. No significant difference was found on total per cent of germination at temperatures 5° to 24°. The main difference was in vigor of germination. Since one of the aims of these trials was to determine the temperature at which secondary sporidia are most frequently produced by primary sporidia lying on pine needles, it was surprising to find that very few were formed at any temperature.

An interesting phenomenon noted with many sporidia was branching of germ tubes and a predominance of 2–3 well developed germ tubes per spore (Fig. 9).

The average results of 3 experiments are presented in Table 2. The figures representing total germination are approximations because of the difficulty of finding all the sporidia on the needles.

Germination of secondary sporidia was tested only at 16°C on sugar pine needles. The sporidia were obtained by placing sections of agar containing primary sporidia over the needles and allowing them to cast secondary sporidia. No direct germination was noted. Here, too, the vigor of germination was typified by branching of germ tubes and predominance of 2–3 germ tubes per spore. The experiment was replicated 8 times. Total germination was in the approximate range of 95–99%, and the longest germ tubes averaged 78.1 μ.

Tests were also conducted to determine if type and vigor of germination differed on needles of different conifer species. Six species of white pines were included in the trials. The tests were run for 24 hours at 15°C. No significant difference was noted in type or vigor of germination on the six white pine species (Table 3). Even though no indirect germination was noted in white pines, it is possible that a few may have been overlooked. The only striking difference between any of the other conifer species was the indirect germination on white fir. Although ponderosa pine appears to stimulate more vigorous germ tubes than the other species, it should be noted that 2–3 germ tubes were produced by sporidia on white pines and that each tube was of equal vigor.

Discussion.—Sporidia of *C. ribicola* are formed under conditions of high humidity and proceed to germinate immediately. Although great numbers of sporidia may be produced on a heavily infected ribes plant, their immediate germination would tend to lessen the number capable of causing infection. Laboratory studies, however, have shown that sporidia are capable of avoiding this exhaustion by the process of indirect germination. The process is a type of perpetuation resulting in the production of secondary, tertiary, etc., sporidia with each successive generation formed at the expense of the preceding one.

TABLE 3.—*Germination of primary sporidia on needles of different conifer species*

Host	% [a] Germ.	% [a] D [b]	Av. LGT [c]	% [a] I [d]
Abies concolor	90	80	63.0 [e]	20
Pseudotsuga menziesii	83	97	67.5 [e]	3
Pinus ponderosa	99	95	105.3 [e]	5
P. lambertiana [f]	99	100	85.0 [g]	0
P. flexilis [f]	99	100	83.6 [g]	0
P. ayacahuite [f]	99	100	88.6 [g]	0
P. monticola [f]	99	100	76.2 [g]	0
P. strobus [f]	99	100	78.0 [g]	0
P. excelsa [f]	99	100	78.1 [g]	0

[a] Approximate percentages.
[b] Direct germination.
[c] Average length of germ tubes (in μ) during direct germination.
[d] Indirect germination.
[e] One germ tube predominates per spore.
[f] White pines.
[g] 2–3 germ tubes predominate and branched; only the longest germ tube was measured.

Arthur (1) considered indirect germination to be an abnormal development that occurs when conditions are not present for normal germination, and he thought of secondary sporidia as being less perfect than primary sporidia. It is questionable whether this is actually abnormal germination. The resultant spores may be slightly smaller but are not less perfect; they are able to produce germ tubes equal in vigor to those produced by primary sporidia and are able to infect sugar pine needles. (The pathogenicity of secondary and tertiary sporidia will be reported in a later paper.) The results presented here point to the conclusion that indirect germination is a means of vegetative perpetuation and an aid in dispersal and longevity of sporidia and that a sporidium, with its ability to germinate either directly or indirectly, can act as an organ of infection or as an organ of sporulation.

Germination *per se* was not found to be an accurate measure of sporidial response. Several other factors had a marked effect, including: type of germination (direct or indirect), vigor of germination (length of germ tubes), the period involved, and the kind and condition of the substrate. A true picture of sporidial response cannot be presented without considering all these factors. For example, germination data suggest that sporidia germinate equally well over a wide range of pH. That this is not true is shown by the type of germination and the length of germ tubes at various pH units. Direct germination was highest at pH 3.0, whereas indirect germination was highest at pH 9.0–10.0. Production of secondary sporidia occurred, however, from pH 4.0 to 11.0. Germination was inhibited at pH 2.0. Secondary sporidia followed the same pH response trend as the primary sporidia.

The data reported herein extend the temperature range of sporidia germination beyond that reported by Hirt (8).

Studies on vigor of germination brought out the need for consideration of the time factor. This factor plays a much more important role under fluctuating field conditions than under controlled conditions as regards duration of optimum meteorological situations for production, dispersal, germination, and infection. The results presented here suggest that a somewhat longer but warmer period will ultimately give as much infection as a shorter, cooler period.

The role of indirect germination in nature is as yet undetermined. It has been shown in preliminary studies that secondary and tertiary sporidia form abundantly on droplets of rain water and therefore could conceivably be formed in flight.

On needles of *Pinus lambertiana*, sporidia germinated from 0.5–1° to 28°C, with no significant difference noted in germination *per se* from 5° to 24°. The prime difference was, again, in vigor of germination. On white pine needles, vigor was manifest not only by the length of germ tubes but also by the fact that 2–3 germ tubes per spore predominated on most of the spores and appeared to exhibit equal vigor. It is not at all uncommon on water agar for a sporidium to produce 2–5 germ tubes per spore; however, only one of these proceeds to elongate. Branching of germ tubes was also common on white pine needles. Very few secondary sporidia were formed on the needles at any temperature. In this case, vigor of germination should be measured by germ tube growth during direct germination, and not by indirect germination. Whatever stimulus the spores are receiving from the host is being utilized in growth of the germ tubes. It may be that here lies support for the argument that indirect germination is a result of the spore's being unable to find a suitable host or substrate.

The predominance of direct germination on needles can be somewhat correlated with the response found in the pH trials. Experiments now in progress show that the pH of a film of water on needles decreases with each successive washing of the needles, whether the wash water used is laboratory-distilled water or rainwater. Engel (6) tested the pH of water collected from various conifer needles and found it to be quite acidic (4.0–4.68).

As with the experiments on time-temperature-pH, an increase in germ tube length was noted on pine needles as the temperature increased to the range 16–20°C, after which the germ tubes began to decrease in length. At 28° they measured 4.2 μ. Germination of secondary sporidia followed the same pattern as that of primary sporidia, exhibiting predominance of direct germination and 2–3 germ tubes per spore.

Germination of primary sporidia on 6 species of white pines showed no significant difference in type or vigor of germination. Variation of different white pine species in susceptibility to the rust is evidently not due to a variation in the suitability of the needle surface for germination of sporidia. This is apparent by comparing germination on *P. excelsa*, a moderately susceptible species, to that on *P. lambertiana*, a highly susceptible species.

LITERATURE CITED

1. Arthur, J. C. 1929. The plant rusts (uredinales). John Wiley and Sons, Inc., New York. 446 p.
2. Bega, R. V. 1959. The capacity and period of maximum production of sporidia in Cronartium ribicola. Phytopathology **49:** 54-57.
3. Clinton, G. P., and F. A. McCormick. 1919. Infection experiments of Pinus strobus with Cronartium ribicola. Conn. Univ. Storrs Agr. Expt. Sta. Bull. **214:** 428-459.
4. Colley, R. H. 1918. Parasitism, morphology, and cytology of Cronartium ribicola. J. Agr. Research **15:** 619-660.
5. DeBary, A. H. 1887. Comparative morphology and biology of the fungi, mycetozoa, and bacteria. Clarendon Press, Oxford. 525 p.
6. Engel, H. 1939. Das verholten der blatter bie benetzung mit wasser. Jahrb. wiss. Botan. **88:** 816-861.
7. Gottlieb, D. 1950. The physiology of spore germination in fungi. Botan. Rev. **16:** 229-257.
8. Hirt, R. R. 1935. Observations on the production and germination of sporidia of Cronartium ribicola. Bull. N. Y. State Coll. Forestry Syracuse Univ. Tech. Publ. 46. 25 p.
9. Reed, H. L., and C. H. Crabill. 1915. The cedar rust disease of apples caused by Gymnosporangium juniperi-virginianae Schw. Virginia Agr. Expt. Sta. Bull. 9. 106 p.
10. Sappin-Trouffy, P. 1896. Recherches histolagiques

sur la famille des urédinées. Botaniste **5**: 59-244.
11. SPAULDING, P., AND ANNIE RATHBUN-GRAVATT. 1925. Longevity of the teliospores and accompanying uredospores of Cronartium ribicola Fischer in 1923. J. Agr. Research **31**: 901-916.
12. SPAULDING, P., AND ANNIE RATHBUN-GRAVATT. 1926. The influence of physical factors on the viability of sporidia of Cronartium ribicola Fischer. J. Agr. Research **33**: 397-433.
13. WEBB, R. W. 1919. Studies in the physiology of fungi. X. Germination of spores of certain fungi in relation to hydrogen ion concentration. Ann. Missouri Botan. Garden **6**: 201-222.
14. WEBB, R. W. 1921. Studies in the physiology of fungi. X. Germination of the spores of certain fungi in relation to hydrogen ion concentration. Ann. Missouri Botan. Garden **8**: 283-341.
15. YORK, H. H., AND W. H. SNELL. 1922. Experiments in the infection of Pinus strobus with Cronartium ribicola. Phytopathology **12**: 148-150.

A PRECISION SPRAY TECHNIQUE FOR EVALUATING OILS FOR SIGATOKA DISEASE CONTROL ON INDIVIDUAL BANANA LEAVES IN THE FIELD

L. Calpouzos, W. A. Brun, T. Theis, and C. Colberg

Plant Pathologist, Plant Physiologist, Assistant Officer in Charge, and Agronomist, respectively.

In cooperation with the Esso Research and Engineering Company, Linden, N. J., and the United Fruit Company, Boston, Mass.

Accepted for publication July 6, 1959.

SUMMARY

A spray technique for applying precise, small amounts of oil to single leaves of banana plants in the field was developed for the evaluation of oils for Sigatoka leaf-spot disease control. Applications of an oil at 1.16, 1.85, and 3.06 gal./A. produced respective median deposits of 1.6, 2.6, and 4.3 mg/100 cm^2 of leaf surface. To reproduce these oil deposits on single leaves, a spray arrangement used an artist's airbrush connected to a tank of compressed air equipped with a reducing valve. The spray oil was delivered to the airbrush by a pipette inserted in the fluid intake. Spray rates were accurate to the nearest 0.01 ml. and the average particle diameter was 48 μ. The oil was directed to the leaf tissue by a cloth-covered shield. The oil deposit on the sprayed area was reproducible.

Evaluation of low-volume oil sprays requires a technique that applies precise, small amounts of oil to single leaves under field conditions. It was recently discovered (2) that mineral oil alone, sprayed in low volumes, controls the Sigatoka leaf-spot disease of bananas whose pathogen is *Mycosphaerella musicola* Leach. Since then, such oil sprays have come into wide use[1] (1) in banana-producing areas; however, little is known of the factors involved in disease control and phytotoxicity. Oil misted at rates as low as 1 gal./A. is sufficient for disease control although such applications deposit very small amounts of oil per unit of leaf area. Critical evaluation of oils requires a spray method that will apply reproducible deposits to individual leaves in quantities comparable to those obtained under commercial spray operations.

Precise laboratory spray techniques for small potted plants have been described (3, 4). These techniques are unsuitable for bananas because of the relatively large size of even young plants. Moreover, it is desirable to obtain data on field plants exposed to a natural environment.

First, this paper presents a determination of the amount of oil deposited on banana leaf surfaces sprayed by a commercial method; and second, details are given of a small-scale technique adapted for depositing precise amounts of spray oil, equivalent to commercial rates, on single banana leaves in the field.

Determination of oil deposit on banana leaves sprayed by a commercial method.—*Materials and methods.*—The test was carried out in a 150×200-ft. plot of mature Dwarf Cavendish plants spaced 5.5×10.0 ft. One hundred and forty-eight squares of aluminum foil, each 10×10 cm, were numbered and weighed individually to the nearest 0.1 mg. After the dew was wiped off the leaves, 1 square was placed at random on the upper surface of each leaf of 12 banana plants located in the center of the plot.

Spraying, carried out when wind velocity was less than 5 mph, was with a back-mounted, gasoline-driven, mist sprayer[2] commonly used on commercial banana farms. According to accepted practice, the operator walked along each row of plants with the spray nozzle pointing straight up. The stream of oil mist, shot 15–20 ft. above the plants, then settled down on the leaves. The oil in the spray tank was measured before and after the application, and the rate computed in gal./A. The oil used was a highly refined mineral oil (#LO-2144, sp. gr. 0.8514, visc. 88.5 S.S.U. at 100°F).[3] Immediately after the plot was sprayed, the aluminum squares were removed, folded to protect the sprayed side, and reweighed in the laboratory. The hands of the collectors were kept free of oil by periodic use of acetone and towels.

No significant evaporation of the test oil from the foil takes place under conditions similar to those during the experiments. This was determined by spraying 5 mg/100 cm^2 on each of 10 aluminum squares. They were weighed immediately and placed outdoors in direct tropical sunlight. One hour later, the squares were reweighed, and no statistically significant change in weight occurred.

[1] Information from United Fruit Co., 80 Federal St., Boston Mass.

[2] Motoblo spraying machine manufactured by the Kent Engineering and Foundry, Ltd., Maidstone, Kent, England.

[3] Supplied by the Esso Research and Engineering Co., Linden, N. J.

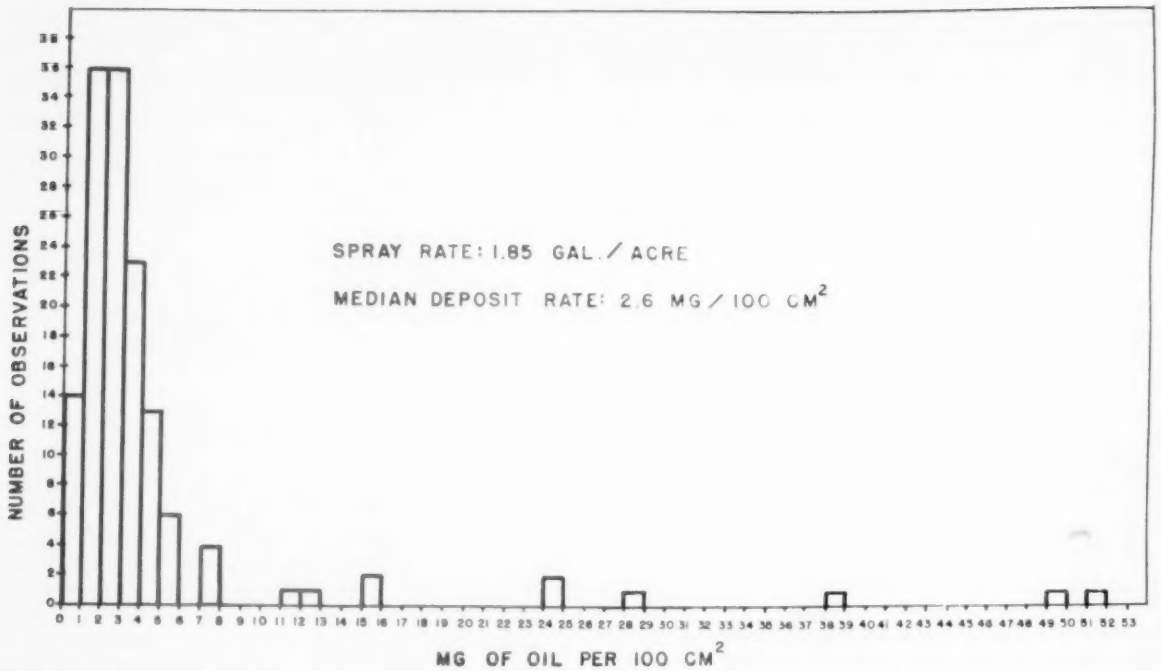


Fig. 1. The weight of oil deposited on 148 squares of aluminum foil attached to banana leaves sprayed with a commercial-type mist sprayer.

Results.—Oil depositions on banana leaves were determined for 3 spray rates—1.16, 1.85, and 3.06 gal./A. Typical results are presented in Fig. 1. The weight of all aluminum squares increased, indicating that the oil-mist reached every leaf sampled. The few cases of high deposits shown in the histogram are probably due to an occasional direct blast of oil mist from the spray nozzle as it passed close to the leaves. Because of the skewness of the results, the median values rather than the means were used. When the median deposition values were plotted against rates of application, they were found to fall on a line intersecting the origin (Fig. 2). This line was used to estimate the median deposition values that would result if other rates were applied with the mist sprayer using the same oil.

The results of the spray tests indicate the deposit range that must be reproduced accurately by a small-scale spray technique for the critical evaluation of oils. Most commercial oil sprays for bananas are applied at the rate of 1–3 gal./A. This range is equivalent to a deposit of 1.4–4.2 mg/100 cm² of leaf area as calculated from Fig. 2. A successful microspray technique must, therefore, be able to deposit these minute amounts of oil on individual banana leaves.

Technique for applying small amounts of oil to banana leaf surfaces.—*Materials.*—The apparatus for the small-scale spray technique consists of an artist's airbrush[4] connected by a thick-walled hose to a reducing valve on a small tank of compressed air (Fig. 3-A). A small gas fitting is attached to the fluid intake on the airbrush (Fig. 3-B). The reducing valve is a standard 2-stage oxygen type. The tank of compressed air is of a size and weight that can be carried conveniently to the field.

A spray shield (Fig. 3-A,D) is used to confine the oil deposit to the leaf area to be sprayed. The shield is shaped somewhat like a megaphone, being 2 ft. tall and having an open base 9¼ in. square. Welded to the top of the shield's frame is a metal collar whose center hole permits the airbrush nozzle to fit snugly into place and point directly at the center of the leaf

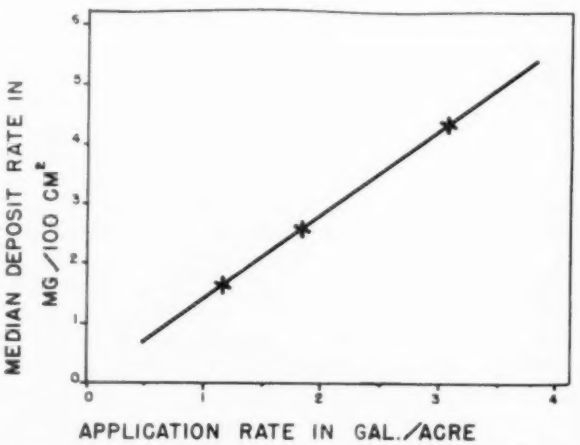


Fig. 2. Spray rate plotted against the median weight of oil deposited per unit area of leaf surface. Three application rates (1.16, 1.85, and 3.06 gal./A.) were tested, using the same oil.

[4] Manufactured by the Paasche Airbrush Co., Chicago, Ill. The number 3 nozzle is used.

section covered by the shield. The shield's frame (Fig. 3-C) is of heavy wire welded together and covered with muslin. A piece of pressboard, 2 ft. square, with a handle attached to the center (Fig. 3-A) supports the leaf, thereby minimizing mechanical injury of the leaf by handling.

Method.—The oil to be sprayed is placed in a 1-ml pipette calibrated in 0.01-ml units. If the diameter of the tip is too small for a satisfactory flow, the point is ground back to a larger size. The tip of the pipette is inserted through the gas fitting, which holds it in place in the fluid intake of the airbrush (Fig. 3-B). Most 1–5-ml pipettes fit snugly in the airbrush intake, thereby preventing oil leakage. The pressure regulator on the tank is adjusted to give a uniform nozzle pressure when the fluid is being sprayed. An arbitrary pressure of 12 lb. per sq. in. was used. Before use, the airbrush is fired briefly to ensure a continuous column of oil from the pipette to the nozzle.

The airbrush nozzle is inserted into the collar of the spray shield, and the square pressboard support is placed under the leaf, which is held as close to horizontal as possible. The shield with the airbrush is rested on the upper leaf-surface (Fig. 3-D), and a predetermined amount of oil is sprayed to the nearest 0.01 ml. Although wisps of sprayfog may escape at the bottom of the shield, the oil deposit is restricted to the leaf area covered by the shield. In actual practice,

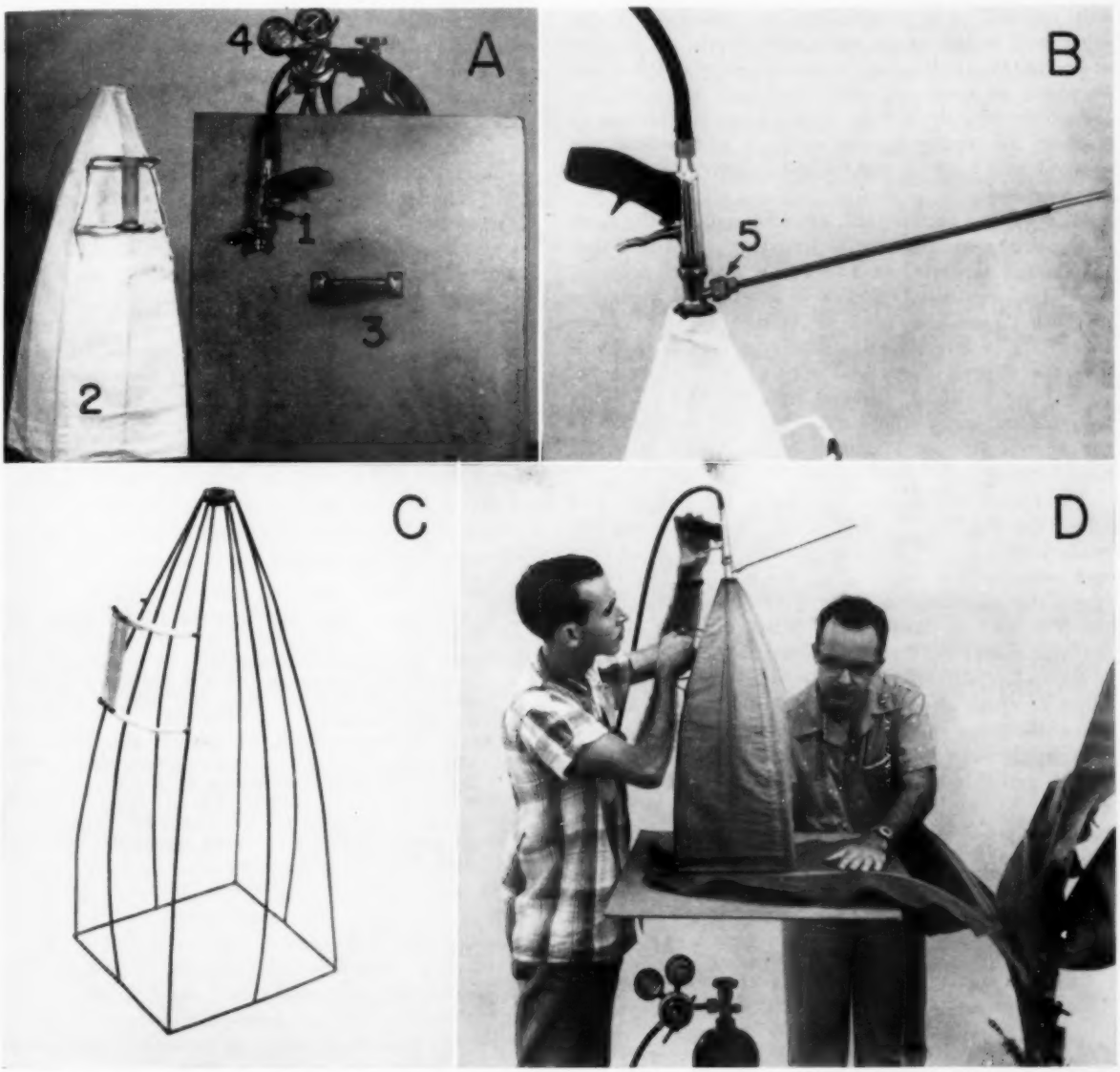


Fig. 3. A) Equipment used for the spray technique: airbrush (1), spray shield (2), pressboard support (3), and tank of compressed air with regulator valve (4). **B)** Closeup of airbrush, with nozzle inserted in the spray shield. The pipette tip is held in place in the fluid intake of the airbrush by means of a gas fitting (5). **C)** The frame of the spray shield. **D)** The equipment being used to spray a banana leaf in the field.

TABLE 1.—*Average weight and standard deviation of replicated oil deposits on 522.6 cm² of wax paper at each of 6 spray rates*

Oil sprayed per shot (ml)	Amount of oil deposited [a]		Estimated spray rate	
	Mg per 100 cm²	Standard deviation	Gallons per acre [b]	Standard deviation
0.05	1.10	0.17	0.78	0.12
0.08	1.89	0.21	1.35	0.15
0.10	2.16	0.19	1.54	0.14
0.30	5.63	0.42	4.01	0.30
0.60	11.91	0.50	8.48	0.36
0.90	17.59	1.13	12.52	0.80

[a] Average of 15 replicates.
[b] Spray rate estimated from Fig. 2.

only one-half of a banana leaf is treated, while the other half is left as an unsprayed check. A number of successive applications along one side of a leaf are necessary to cover an entire leaf-half, since banana leaves are usually 3–6 ft. long. After completion of a given oil treatment, the airbrush and pipette are cleaned with acetone and flushed briefly with the next oil to be used.

Comparisons between oils must be made on the basis of equal deposits per unit leaf area. It was found that the amount deposited on leaves may vary with different oils when any fixed volume is discharged from the airbrush. Therefore, each oil is first sprayed in the laboratory to determine the discharge volume that will give the deposit desired. Pieces of wax paper 9 in. square are weighed, sprayed with the test oil using the airbrush-spray shield assembly, and reweighed to the nearest 0.5 mg 1 minute later. A new paper is used for each subsequent spray.

The size of the pipette opening at the delivery end affects the quantity of oil deposited. A comparison was made with a given oil using pipettes of the same type and capacity but differing in the size of their openings: the resulting deposits differed statistically at the 5% level of significance. When different oils are sprayed, therefore, the same pipette should be used.

Reproducibility of spray deposits.—The reproducibility of spray deposits of oil no. LO-2144 at several given discharge rates was measured in the laboratory. One application at each of 6 discharge rates was tested, and this was repeated 15 times. Table 1 summarizes the results obtained at each discharge rate in terms of mg deposited/100 cm² and also as estimated gal./A. that would give such a deposit. The latter were calculated from the curve in Fig. 2. The reproducibility of spray deposits obtained is indicated by the standard deviations. These deviations become insignificant when compared with the variations observed on the sprayed leaves in the field (Fig. 1).

Distribution of the deposit and particle size.—The uniformity of the oil deposit was measured on the sprayed area covered by the shield. Squares of aluminum foil, 6×6 cm, were numbered and weighed individually. Five positions were selected within the area covered by the shield, i.e., the center and each of the 4 corners. An aluminum square was placed in each of the 5 positions, the whole area was sprayed with 0.1 ml oil, using the airbrush, and the squares were then reweighed to determine the oil deposit. This test was replicated 10 times. The average oil deposit for each of the corner positions was 1.79, 1.97, 1.99, and 2.07 mg/36 cm² while the center position received an average of 2.56 mg. The amount of oil deposited in the center of the sprayed surface was significantly higher at the 1% level than the areas near the corners; among the latter there were no significant differences in oil deposit.

The droplet size was estimated by using glass slides placed at the 5 positions under the shield and sprayed. Within 5 minutes of being sprayed, the slides were examined microscopically using an ocular micrometer for measuring. No noticeable evaporation occurred during the period of observation. The extremes in droplet diameter were 13 and 136 μ. The averages for the 5 positions ranged from 44 to 52 μ., with an over-all average of 48 μ. These figures fall in the lower part of the range of droplet diameters obtained from the commercial-type mist sprayer, where the extremes were 26 and 473 μ., with an over-all average of 181 μ.

—Federal Experiment Station, Agricultural Research Service, United States Department of Agriculture, Mayaguez, Puerto Rico.

LITERATURE CITED

1. ANONYMOUS. 1959. The sigatoka slayer. Lamp **41**: 6-9.
2. GUYOT, H., AND J. CUILLE. 1955. Les traitements fongicides des bananeraies. II. Efficacités des différents modes de traitements. Rôle de l'huile. Fruits outre mer **10**: 101-107.
3. SHAW, W. C., AND C. R. SWANSON. 1952. Techniques and equipment used in evaluating chemicals for their herbicidal properties. Weeds **1**: 352-365.
4. TEN HOUTEN, J. G., AND M. KRAAK. 1949. A vertical spraying apparatus for the laboratory evaluation of all types of liquid pest control materials. Ann. Appl. Biol. **36**: 394-405.

INHERITANCE OF RESISTANCE TO POTATO VIRUS Y DERIVED FROM TWO STRAINS OF CAPSICUM ANNUUM

A. A. Cook and C. W. Anderson

Accepted for publication July 8, 1959.
Florida Agricultural Experiment Station Journal Series, No. 890.

SUMMARY

Resistance to potato virus Y, derived from 2 different strains of *Capsicum annuum* designated P11 and S.C. 46252, was found to be inherited as a single recessive factor denoted by the symbol y^a. Both resistant pepper strains were homozygous for the recessive allele. The susceptible varieties California Wonder, Florida Giant, Improved Worldbeater, and Yolo Wonder were homozygous for the dominant allele Y^a.

Potato virus Y (PVY) has been found on bell peppers (*Capsicum annuum* L.) (9) throughout Florida and has been especially destructive in the southern part of the state (1, 8).

Relatively little has been reported on the inheritance in pepper of resistance to PVY. The common pepper viruses of Puerto Rico (7) and Trinidad (3) were similar, and 1 of them was serologically related to PVY (5). Both strains differ from those found on pepper in Florida (2). The resistant variety Puerto Rico Wonder was developed from the resistant Cuaresmeño variety, but both showed mild symptoms when infected with the Trinidad strain (3, 6). Resistance from Cuaresmeño was inherited as a "single genetic factor" (6). In contrast, the PVY strains studied in Florida caused no symptoms on resistant varieties P11 and S.C. 46252 (2). It is known (personal correspondence with P. V. V. Weber and J. N. Simons) that there are other pepper varieties resistant to certain PVY strains found in the United States.

Following the discovery (2) that varieties P11 and SC (S.C. 46252) were resistant to 3 viruses (PVY, tobacco etch virus, and tobacco mosaic virus), a study of the genetics of the resistance of these 2 varieties to PVY was undertaken. Other workers have previously studied the inheritance in pepper of resistance to the tobacco etch and tobacco mosaic viruses.

Materials and methods.—Virus strains previously designated PVY-N and PVY-C (2) were used.

The resistant peppers P11 and SC were crossed with the susceptible varieties CW (California Wonder), FG (Florida Giant), IWB (Improved Worldbeater), and YW (Yolo Wonder). Backcrosses were accomplished in the F_1 generation.

Test populations were inoculated by the carborundum method of mechanical inoculation. With the exception noted below, all plants that did not develop symptoms were reinoculated 2–3 weeks after the first inoculation and observed for an additional 21–30 days. Tabasco pepper plants (*Capsicum frutescens* L.) were inoculated to check virus infectivity and identity.

Attempts were made to recover virus by grafting scions from resistant graft-inoculated F_2 plants onto healthy susceptible pepper stocks 32–63 days after inoculation.

Except where noted, a population included all the plants derived from seed of a single fruit.

Results.—*F_1 inoculations.*—The results of inoculation of F_1 progenies are listed in Table 1. There are some apparent discrepancies in that not all plants were recorded as susceptible, but 2 explanations are offered. First, no F_1 test plants were reinoculated, and the single plant that failed to exhibit symptoms following inoculation with PVY-N was probably a skip. Second, the 39 plants listed as uninfected after inoculation with PVY-C were probably read erroneously. They were the first F_1 plants to be inoculated, and developed (after an unusually long incubation period) a type of mild mottle not attributed to PVY-C at the time. All 6 attempts to recover virus from uninoculated parts of these plants were successful, and all F_1 plants subsequently inoculated with PVY-C were obviously diseased. All of the recovery attempts from uninoculated parts of F_1 plants inoculated later were successful.

F_2 and F_3 inoculations.—Table 2 shows that the ratio of susceptible to resistant plants among various F_2 populations was about 3:1. All but a single F_2 population contained more than 16 plants, a number sufficient to ensure the occurrence of at least 1 homozygous recessive genotype with a probability of 99:1 (4). The only significant deviation ($P = <0.05$) from the 3:1 ratio occurred among the IWB×P11 populations. Consequently, resistance to PVY-N appears correlated with the homozygous condition of a single recessive gene herein designated y^a.

Infected scions were cleft grafted onto each resistant stock of 2 F_2 populations. None of 4 CW×P11 and 20 CW×SC stocks developed symptoms after graft inoculation, and scions taken from each of these resistant stocks failed to transmit PVY-N to healthy YW

TABLE 1.—*Results of mechanical inoculation of F_1 progenies with two strains of potato virus Y*

Pedigree of test plants	PVY-N		PVY-C	
	Number of plants		Number of plants	
	Inoculated	Infected [a]	Inoculated	Infected [a]
CW × P11	12	12	31	11
FG × P11	10	10	29	10
IWB × P11	21	20	19	19
YW × P11 [b]	24	24	44	44
Total	67	66	123	84
CW × SC	16	16	6	6
FG × SC	10	10		
IWB × SC	4	4		
YW × SC	18	18	9	9
Total	48	48	15	15

[a] Number of plants visibly infected.
[b] Test plants from appropriate reciprocal crosses included.

TABLE 2.—*Results and χ^2 analysis (3:1) of mechanical inoculation of F_2 populations with PVY-N*

Pedigree of F_2 hybrids	Number of plants		Degrees of freedom	χ^2 analysis			
	Susceptible	Resistant		Total	Pooled	Heterogeneity	P
CW × P11	106	42	5	3.669			.5–.7
			1		0.900		.3–.5
			4			2.769	.5–.7
FG × P11	117	28	4	5.616			.2–.3
			1		2.504		.1–.2
			3			3.112	.3–.5
IWB × P11	154	54	5	11.304			.02–.05
			1		0.104		.7–.8
			4			11.200	.02–.05
YW × P11	231	86	8	7.692			.3–.5
			1		0.768		.3–.5
			7			6.924	.3–.5
Total	608	210	25	28.281			.2–.3
			1		1.972		.1–.2
			24			26.309	.3–.5
CW × SC	198	62	5	4.452			.3–.5
			1		0.184		.5–.7
			4			4.268	.3–.5
IWB × SC	124	56	4	5.304			.2–.3
			1		3.584		.05–.10
			3			1.720	.5–.7
YW × SC	128	50	4	3.584			.3–.5
			1		0.908		.3–.5
			3			2.676	.3–.5
Total	450	168	15	13.340			.5–.7
			1		1.572		.2–.3
			14			11.768	.5–.7

stocks. These results provide additional evidence that the resistant F_2 plants were as resistant as the resistant parents and that the recessive gene y^a severely limits or entirely prevents virus multiplication when homozygous. Consequently, the effect of $y^a y^a$ cannot be attributed to prevention of virus establishment following sap inoculation alone and must be attributed to the limiting effect on virus multiplication.

As a further test of the single-recessive theory of resistance, a number of susceptible and resistant F_2 plants were allowed to set seed and the resulting F_3 populations inoculated (Table 3). All resistant F_2 plants produced only resistant progenies. Within the limits of experimental error, one-third of the susceptible F_2 plants produced only susceptible seedlings and two-thirds yielded segregating F_3 populations.

Backcross populations.—With the exception of 2 cases, the F_1 backcross data (Table 4) substantiate the single-recessive theory of resistance. The first exception [(FG × SC) × FG] was apparently an F_1 self. The second [(SC × YW) × SC] was a significant deviation from the expected ratio explained as a chance occurrence.

Discussion.—Although the recessive factor y^a confers resistance when homozygous, present information is insufficient to determine whether or not the recessive gene from P11 is identical with that from SC. However, no differences in response to PVY were noted between resistant plants derived from P11 and those from SC.

TABLE 3.—*Genotypic analysis of some susceptible and resistant F_2 plants by mechanical inoculation of F_3 populations* [a]

Varietal cross from which F_2 plants were derived	Number of F_2 plants whose progeny were tested		Genotypes of F_2 plants as derived from progeny tests		
	Susceptible	Resistant	YY	Yy	yy
CW × P11	7	2	2	5	2
FG × P11	7	2	5	2	2
IWB × P11	5	2	1	4	2
YW × P11	5	2	0	5	2
Total	24	8	8 [b]	16 [b]	8 [b]
CW × SC	7	2	2	5	2
IWB × SC	7	3	1	6	3
YW × SC	7	2	1	6	2
Total	21	7	4 [c]	17 [c]	7 [c]

[a] Populations consisted of 8–101 (average 33) F_3 seedlings from each F_2 plant.
[b] $\chi^2 = 0.000$; P = .99.
[c] $\chi^2 = 1.929$; P = .3–.5.

TABLE 4.—*Results and χ^2 analysis (1:1 and 1:0) of mechanical inoculation of F_1 backcross progenies with PVY-N*

Pedigree of backcross plants	Number of plants		Degrees of freedom	χ^2 analysis			
	Susceptible	Resistant		Total	Pooled	Heterogeneity	P
(CW × P11) × P11	44	43					
(FG × P11) × P11	25	32					
(FG × P11) × P11	30	29					
(YW × P11) × P11	29	34					
Total	128	138	3	1.284			.7–.8
			1		0.376		.5–.7
			2			0.908	.5–.7
(SC × YW) × SC	30	48		4.154			.02–.05
(CW × P11) × CW	55	0					
(IWB × P11) × FG	74	0					
(YW × P11) × YW	5	0					
Total	134	0	2	0.000			.99
			1		0.000		.99
			1			0.000	.99
(CW × SC) × FG	53	0					
(FG × SC) × FG	28 [a]	7 [a]					
(SC × YW) × YW	130	0					
Total	183	0	1	0.000			.99
			1		0.000		.99

[a] Not included in χ^2 analysis.

Other peppers resistant to PVY strains are known, but comparable genetic studies using identical PVY isolates have not been reported. Differences in PVY strains used and in reported reactions of resistant plants also prevent comparison of the recessive gene y^d with the "single genetic factor" responsible for resistance in Cuaresmeño pepper and its derivative, Puerto Rico Wonder (3, 6).

The reliability of the mechanical inoculation technique used in the present study was supported by the fact that more than 99% of the susceptible plants (which varied in age at inoculation) were consistently infected by the first inoculation. Moreover, after reinoculation, limited graft inoculations failed to reveal the presence of additional susceptible plants.—Florida Agricultural Experiment Station, Gainesville.

LITERATURE CITED

1. ANDERSON, C. W., AND M. K. CORBETT. 1957. Virus diseases of peppers in central Florida. Survey results 1955. Plant Disease Reptr. **41**: 143-147.
2. COOK, A. A., AND C. W. ANDERSON. 1959. Multiple virus disease resistance in a strain of Capsicum annuum L. Phytopathology **49**: 198-201.
3. DALE, W. T., 1956. Virus diseases of solanaceous crops in Trinidad. Trop. Agr. (Trinidad) **33**: 35-50.
4. KNIGHT, R. L. 1948. Dictionary of genetics, including terms used in cytology, animal breeding, and evolution. Chronica Botanica Co., Waltham, Mass. 183 p.
5. PÉREZ, J. E., AND J. ADSUAR. 1955. Antigenic relationship between Puerto Rican pepper-mosaic virus and a strain of potato virus Y. J. Agr. Univ. Puerto Rico **39**: 165-167.
6. RIOLLANO, A., J. ADSUAR, AND A. RODRIGUEZ. 1948. Breeding peppers resistant to a Puerto Rican type of mosaic. Proc. Am. Soc. Hort. Sci. **51**: 415-416.
7. ROQUE, A., AND J. ADSUAR. 1941. Studies on the mosaic of peppers (Capsicum frutescens) in Puerto Rico. J. Agr. Univ. Puerto Rico **25**: 40-50.
8. SIMONS, J. N., R. A. CONOVER, AND J. M. WALTER. 1956. Correlation of occurrence of potato virus Y with areas of potato production in Florida. Plant Disease Reptr. **40**: 531-533.
9. SMITH, P. G., AND C. B. HEISER, JR. 1951. Taxonomic and genetic studies on the cultivated peppers, Capsicum annuum L. and C. frutescens L. Am. J. Botany **38**: 362-368.

ONION BLAST OR LEAF SPOTTING CAUSED BY SPECIES OF BOTRYTIS

R. H. Segall and A. G. Newhall

Formerly Research Assistant, Department of Plant Pathology, Cornell University, Ithaca, New York; now Plant Pathologist, United States Operations Mission to Costa Rica, International Cooperation Administration.

Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, New York.

A condensed version of a thesis submitted by the senior author in partial fulfillment for the Ph.D. degree.

Accepted for publication July 6, 1959.

SUMMARY

A disease of onions known as blast was found to be caused by several species of *Botrytis*, the commonest of which is *B. allii*. Spores germinating on the surface of onion leaves caused spotting typical of blast if the plants were incubated in a humid atmosphere and received enough light. Shortly after appearance of the leaf-spotting phase the tips die back and the plants may die prematurely. There is no evidence of cuticular or stomatal penetration by the germinating spores, and hyphae were not found in the lesions. When the fungus was grown in nutrient broth plus dextrose, a toxin was produced capable of causing leaf spots indistinguishable from those induced by the germinating spores of *Botrytis*. The disease was controlled by spraying onions at weekly intervals with various protective fungicides, such as nabam, ferbam, and captan, all of which significantly reduced the number of leaf spots caused by this *Botrytis*, and subsequent blighting.

A disease known as "blast" has frequently caused serious losses to onions grown in New York and other states. Other names for the disease include "blight," "tip burn," and "tip blight." Symptoms resulting from blast may be confused at times with those caused by thrips, drought, or excessive soil moisture, all of which can cause eventual dieback. But in blast such dieback is always preceded by characteristic leaf spotting.

The first report of symptoms similar to those of blast was made by Whetzel (8), who described onion leaves becoming white and withered 1–3 in. down from the tips. No mention was made of the leaf spotting phase of the disease. Whetzel thought the cause was wet weather and poor soil drainage. The leaf spotting was first mentioned by Clinton, in Connecticut (1), who described yellowish spots on leaves as caused by a *Botrytis*. He later decided that no fungus was responsible for blast (2). Munn (5) also reported *B. allii* Munn as pathogenic on onion leaves. Doran and Bourne (3) and Jones (4) concluded that the disease was of a physiological nature. Jones made a study of the environmental factors contributing to blast and concluded that the symptoms were the result of excess water loss through transpiration, resulting in death of part of the leaf tissue. That *Botrytis* might have been present in his greenhouse chambers is an interesting possibility.

Symptoms.—Blast can be described as a two-phase phenomenon: 1) a leaf flecking or spotting, followed by 2) a dieback of the tips, proportional in extent to the severity of the spotting, which after a few days of warm, humid weather may result in almost complete chloronemia[1] and blighting of the foliage. The spotting phase is illustrated in Fig. 2, and the final stage in Fig. 1. Many spots develop green halos that persist as the leaves become chloronemic.

Fig. 1. Variety Yellow Globe, midseason, unsprayed, showing abundant leaf spotting and some dieback symptoms both typical of "blast."

In the field, blast usually occurs when the foliage

[1] Yellowing "due to degeneration or disorganization of chlorophyl or chloroplasts" as distinct from "chlorosis . . . due to failure of development of green color in chlorophylous tissue." (Whetzel's terminology)

area is at its maximum, before the bulbs have enlarged appreciably and while the necks are still succulent. Bulbs therefore remain small and necks remain soft. When such onions are harvested and the tops removed, these succulent necks provide an excellent infection court for neck- and bulb-rotting organisms. Thus *Botrytis* neck rot in stored onions is very likely to be more severe following seasons in which blast is

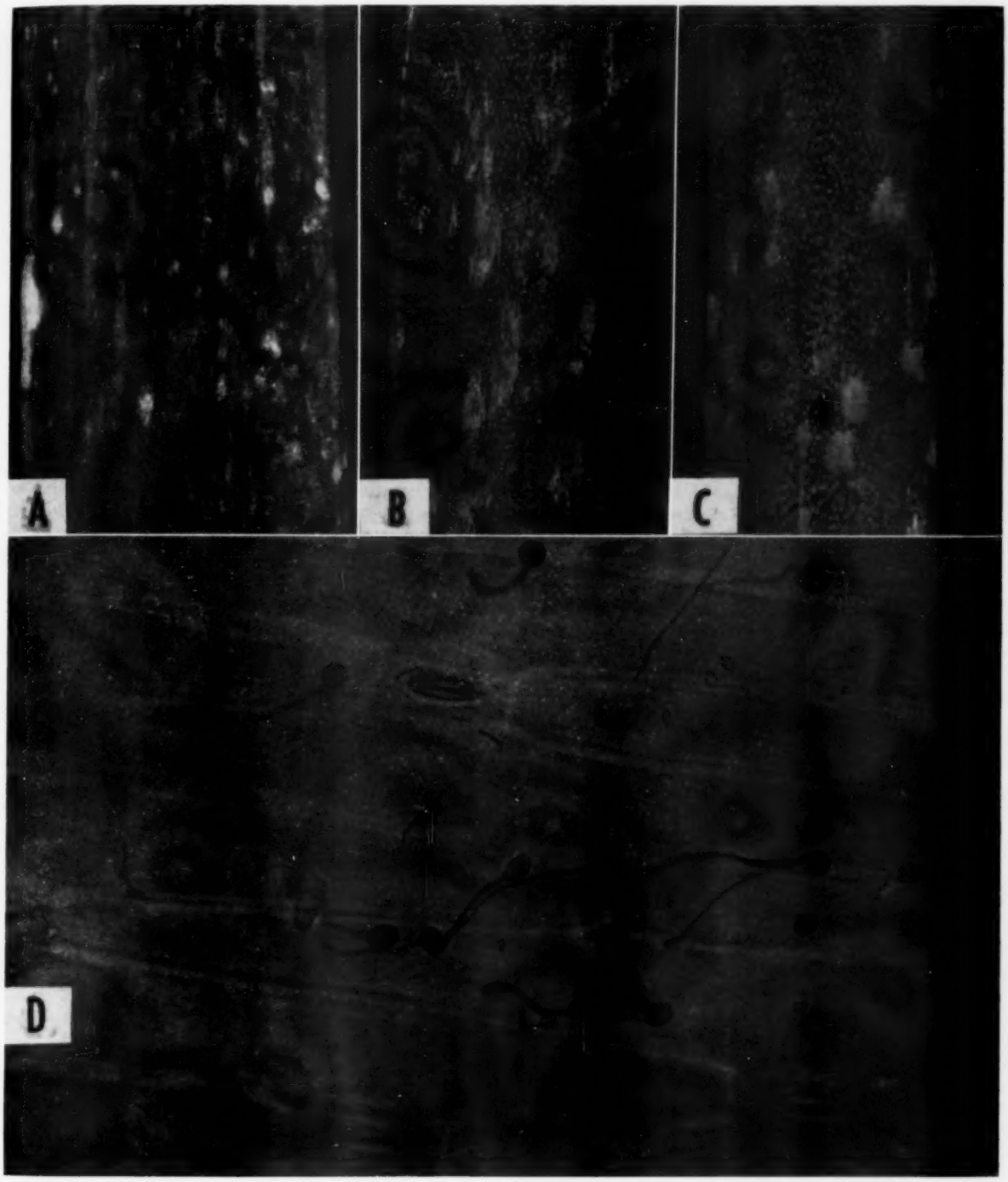


Fig. 2. A) Onion leaf (×8) showing typical natural spotting. **B)** Spotting resulting from spraying leaf with fungus freed filtrate of a four-day-old nutrient glucose broth culture of *Botrytis allii*. **C)** Similar spotting from a water suspension of *Botrytis allii* spores. **D)** Germinating spores of *Botrytis cinerea* on surface of onion leaf, cleared in saturated chloral hydrate and stained in .05% acid fuchsin in lactophenol; showing no penetration of the host, either cuticular or stomatal. (×254).

prevalent.

Tests on cause of blast.—Work by Clinton (2) and Jones (4) showed that the occurrence of blast is correlated with certain weather conditions. Accordingly detailed records of air and soil temperature, relative humidity (RH), rainfall, and water table were kept in Orange County muckland onion fields for 4 growing seasons.

Air humidity was found critical for the leaf spotting phase, and air temperature is important for the leaf blighting phase. Leaf spotting occurs when RH is close to 100% for at least 24 hours. Leaf blighting occurs after the leaf spotting phase and requires temperatures above 80°F. Presumably, these two factors respectively favor spore germination and rapid transpiration.

To study the effect of high RH on onions, a shelter of cloudy cellulose acetate, 50×40×40 in., was placed over onions growing in the field, covering them completely. Water was then sprayed into the chamber continuously for periods up to 36 hours using a tank of oxygen as a source of pressure. While the onions were sprayed the RH under the shelter ranged from 70 to 100%. At the close of the 36-hour period many newly formed leaf lesions were found on leaves of onions atomized with this water. Leaves of plants not enclosed and therefore not subjected to artificially high humidity did not show any additional spotting. High humidity is evidently one necessary factor in leaf spotting.

It was noted that conidiophores of *Botrytis* were commonly associated with senescent onion leaf tips. Leaves of onions growing in the field were accordingly inoculated by spraying with spore suspensions prepared from pure cultures of this *Botrytis*. Check plants were sprayed with water. Both inoculated and check plants were then covered with moist chambers made of unpolished cellulose acetate and incubated for 48–72 hours. The inoculated plants developed a high incidence of spotting typical of the leaf spot phase of blast; the check plants showed only occasional isolated spots. The presence of *Botrytis* spores is evidently another very important factor in the production of leaf spotting.

The effect of high humidity on sporulation of *B. allii* was demonstrated later in the laboratory by inoculating dried, healthy onion leaves with a spore suspension and suspending these leaves over various concentrations of sulfuric acid in air-tight chambers. In 3 days, conidiophores bearing spores developed in saturated atmosphere, and in 5 days they were evident at every RH down to 80%.

The effect of light on this spotting phase was studied next. Onion plants grown in the greenhouse were inoculated with a spore suspension of *B. allii* and placed in a specially constructed moist chamber in which humidity was maintained close to saturation by water constantly running down the sides of the chamber. In some experiments the chamber consisted of 36-gal. covered pails with water in the bottom. After incubation periods of 24–72 hours in the dark, only a few leaf spots appeared, and only on older leaves. However, on plants inoculated and then enclosed in light-transmitting polyethylene bags and incubated 72 hours in the open greenhouse, the spots developed were similar in both quantity and appearance to spots resulting from field inoculations under normal light conditions. Control plants enclosed in bags and sprayed with water only, and plants inoculated but not covered, showed no leaf spotting. Furthermore, plants covered with polyethylene bags but incubated in the dark showed no leaf spotting. It was thus demonstrated that the leaf spotting phase of onion blast occurred only when leaves of onions inoculated with *Botrytis* spores were incubated under conditions of high RH and strong light.

Spore load and extent of leaf spotting.—The concentration of a series of spore dilutions in water was measured with a Fuchs-Levi counting cell. A 50-ml aliquot of each concentration was then sprayed onto each of 4 onion plants of about equal size. The plants were covered with polyethylene bags and incubated 48 hours in the greenhouse and leaf spot counts then made (Table 1).

Not only is there a fairly straight-line relation between spore load and number of spots but the individual spots were larger where the concentration of spores was the highest. This is confirmatory evidence of toxin production by germinating spores of *Botrytis* as the cause of leaf spotting.

Inoculum sources and viability.—When it had thus been established that *Botrytis* was a cause of spotting of onion leaves, attention was turned to the source of this inoculum. Although *Botrytis* conidia and conidiophores could not be found on individual leaf spots, they were found in many fields in profusion on necrotic areas of onion leaves and on older dead leaves throughout the growing season. During periods of high soil moisture they were found on outer senescent leaves and scales of small growing bulbs. They were also abundantly fruiting on cull onions discarded the previous year. The fungus was also isolated from commercial onion sets, but not from seed. It is believed, though not yet proved, that in damp, humid, warm weather, great showers of *Botrytis* spores may occur in many onion fields. Later, in greenhouse tests, *Botrytis* spores sprayed onto leaves of potted onion plants could apparently retain their viability or ability to cause spotting for up to about 8 days (Table 2).

TABLE 1.—*Relation of spore concentration to number of spots per leaf*

No. spores/ml	Mean no. spots per plant
591,250	222
371,290	128
106,850	24
40,000	9
12,900	8
6,678	4
1,094	0
0	0

TABLE 2.—*Duration of viability of spores on dry, living onion leaves*

Treatment after spraying plants with spore suspension and drying before a fan	Mean spots per plant
1. Incubated while still wet for 48 hours	158
2. Fan dried before incubation	170
3. Fan dried and held 24 hours before incubation	240*
4. Fan dried and held 48 hours before incubation	102
5. Fan dried and held 4 days before incubation	115
6. Fan dried and held 6 days before incubation	126
7. Fan dried and held 8 days before incubation	28*
8. Fan dried and held 10 days before incubation	0
9. Fan dried and held 12 days before incubation	0
LSD 19:1 87.6*	
LSD 99:1 120.4**	

During this time any 48-hour incubation period at high humidity in light resulted in much spotting, but after the eighth day none occurred. The optimum length of the incubation period was later found to be in the neighborhood of 24 hours. Small spots could be observed after 22 hours in the humidity chamber, and larger spots occurred after 26 hours. Further incubation produced no appreciable changes.

Species of Botrytis capable of causing blast.—All the isolates of *Botrytis* found associated with onion blast in the field corresponded to *Botrytis allii* Munn. Of the species causing neck rot, this is the one found to sporulate most abundantly (7). However, other species of *Botrytis* used in inoculation experiments, such as *B. cinerea*, *B. tulipae*, and *B. paeoniae*, also caused symptoms similar to those caused by *B. allii*. But no spots or other symptoms were noted on onion leaves inoculated with spores of other genera including *Stemphylium*, *Alternaria*, *Colletotricum*, and *Cephalosporium*.

Histological studies of leaf spots.—*Botrytis* was never successfully isolated from naturally occurring leaf spots in the field. And even though it was possible to reproduce the leaf-spotting symptoms of blast by inoculation with *Botrytis* spores, the fungus still could not be reisolated if the leaves were surface-sterilized with such chemicals as sodium hypochlorite, mercury bichloride, or phenol. Accordingly, a histological study of diseased tissue was made to determine the mode of penetration of the causal organism, if any. Small portions of leaves containing spots were taken from plants naturally and artificially inoculated in the field and greenhouse. These leaf fragments were cleared in chloral hydrate for 12–24 hours and then stained in 0.05% acid fuchsin in lactophenol for 12 hours. The acid fuchsin stained fungus tissue red, but did not stain the cleared leaf tissue. In a second method, an epidermal strip was carefully removed from the portion of the leaf containing the spot, placed on a microscope slide, and flooded with 0.05% acid fuchsin in lactophenol.

Examination of the dead and stained lesions and of stained epidermal strips revealed germinating *Botrytis* spores only on the surface of the lesions (Fig. 2-D). No penetration by the germ tubes, through either cuticle or stomatal opening, could be found, nor any mycelium in the leaf tissue. The germ tubes on the leaf surface did not grow in any particular direction with relation to the position of the stomata, and in several cases were seen to have grown over the stomatal openings without entering them.

In the lesions themselves, the epidermal cells became separated from the palisade cells below the epidermis, with both the palisade cells and the parenchyma cells becoming completely disorganized. A separation of cell walls was noted, as if the middle lamella had been dissolved.

Leaf fragments containing spots taken from plants naturally inoculated under field conditions showed germinating spores similar in appearance to *Botrytis* on the lesion surfaces in about one-third of the sections examined. Alternaria-type spores were also found. But no mycelium was found, except in old lesions that had become necrotic. This septate mycelium was believed to belong to some secondary invader.

The accuracy of the histological technique just described, which failed to reveal the presence of the fungus in the leaf lesion, was checked by the following procedures. It had been found by Williamson (unpublished), working on tulip leaf spot caused by *Botrytis tulipae* (Lib.) Lind, that germ tubes penetrated the cuticle, and mycelium was present in the infected cells. Therefore, tulip leaf sections containing typical leaf spots were cleared and stained by the method used on onions. These cleared and stained tulip leaves showed germinating *Botrytis* spores on the surface of the lesions, with germ tubes invading infected cells. In a second procedure, onion leaf sections were boiled in water for 10 minutes to kill the tissue, which was then seeded with a spore suspension of *B. allii* and incubated for 48 hours in a moist chamber. The tissue was then cleared and stained in the usual manner. Microscopic examination revealed germ tubes on the leaf surface, penetrating the epidermal cells, and mycelium growing throughout the tissue. Therefore, the clearing and staining procedure used with onion leaves is considered reliable, and the lack of penetration of the living leaves, demonstrated by the method, is significant.

The relationship of light to blast.—Since leaf-spotting symptoms were more severe when plants were inoculated under conditions approximating daylight, the effect of light on leaf spotting was further tested. Twelve greenhouse-grown onion plants were inoculated with a spore suspension of *B. allii* and immediately covered with semitransparent polyethylene bags. Kraft-paper bags were placed over 6 of these to exclude light. In addition, inoculated onion plants were covered with polyethylene bags and placed in constant-temperature chambers some of which were each illuminated by ten 30-watt fluorescent bulbs. Inoculated plants were exposed to: 1) continuous light for 60 hours, 2) 12 hours of light alternated with 12 hours of darkness for 60 hours, and 3) continuous darkness for 60 hours. Plants were also exposed under these three light conditions at three temperatures: 13, 18,

TABLE 3.—*Effect of manipulating the two environmental factors of temperature and light on incidence of leaf spotting*

Treatment	Environmental changes during 60-hour incubation	Mean spots per plant
1	Constant temp. (24°C)—continuous light	13
2	Constant temp. (13°C)—continuous light	60
3	Constant temp. (24°C)—continuous darkness	4
4	Constant temp. (13°C)—continuous darkness	3
5	Constant temp. (24°C)—alternate 12-hr. light and 12-hr. dark	79
6	Constant temp. (13°C)—alternate 12-hr. light and 12-hr. dark	54
7	Constant light—alternate 12-hr. periods at 13 and 24°C	24
8	Constant darkness—alternate 12-hr. periods at 13 and 24°C	32 [a]
9	Alternate between darkness at 13 and light at 24°C	30
10	Alternate between light at 13 and darkness at 24°C	58

[a] No accounting for the apparent discrepancy between this result and numbers 3 and 4.

and 24°C.

Inoculated plants incubated in the greenhouse for 72 hours covered only with polyethylene bags had an average of 164 spots per plant, whereas plants covered with polyethylene and kraftpaper bags to exclude light averaged 43 spots per plant. The numbers of spots on plants incubated in constant-temperature chambers under various periods of artificial light are given in Table 3. Spotting of onion plants inoculated with a spore suspension of *B. allii* occurs after incubation periods of 60 hours, both in continuous light and in alternating light and darkness. Inoculated plants incubated in darkness for 60 hours had very few spots. Why light is necessary was not determined.

Toxin production by botrytis allii.—*Materials and methods.*—Spores of *B. allii*, formed by the fungus growing on potato-dextrose agar, were aseptically placed in liquid-culture solutions. The media used were modified Richard's solution, modified Czapek's solution, potato dextrose broth, and nutrient broth plus dextrose. The resulting cultures were incubated, either in a shaker or incubator, at 21°C. Mycelium and spores were removed, following incubation for various periods, by passing the culture solution through fine-grade sintered glass or Seitz bacteriological filters. To assay for toxin production, the resulting filtrates were sprayed immediately on leaves of onion plants that were then covered with polyethylene bags and placed in the greenhouse or in constant-temperature chambers.

To measure mycelial growth of *B. allii* in relation to toxin production, mycelium was removed from the culture solution by passing the solution through Gooch crucibles before passing the filtrate through sintered glass filters in the usual manner. The mycelium was dried to a constant weight at 100°C. The fungus filtrate was then sprayed on potted onion plants to test for toxin content.

In determining the effect of dextrose concentration on toxin production the dextrose solutions were sterilized by passing through a Seitz filter before being added to the autoclaved nutrient broth.

TABLE 4.—*Relation of temperature to growth of mycelium in nutrient broth plus glucose, and to toxin production, as measured by number of spots per onion plant*

Temperature	Dry weight of mycelium produced (mg)	Average no. of spots per plant [a]
12°C	104	29
15°C	166	69
18°C	218	259
21°C	292	363
24°C	240	371
27°C	201	105
30°C	92	18

Correlation coefficient 0.912. Highly significant.

[a] 5 plants per treatment.

Growth of the fungus was good in potato dextrose broth and on nutrient broth plus dextrose. Growth was slight on synthetic Czepek's and Richard's solutions.

Tests with sterile filtrates.—Leaf spotting occurred only on plants sprayed with the filtrate of the fungus grown on nutrient broth plus dextrose. The spots produced by the filtrate were very similar to those formed on the onion leaves by the germinating *Botrytis* spores except for greater variation in size and shape (Fig. 2-B, C). Plants sprayed with fungus filtrates of other culture solutions showed no spotting symptoms. Maximum toxin production occurred 4–6 days after the nutrient solution was seeded with spores of *B. allii*. Heating the fungus filtrate 10 minutes at 100°C or keeping the fungus filtrate 12 hours at room temperature inactivated the toxin.

By growing *B. allii* in nutrient solutions for 5 days at 12, 15, 18, 21, 24, 27 or 30°C, a relationship between mycelial growth and toxin production was obtained (Table 4). There was a high positive correlation between growth of *B. allii* in culture and the amount of leaf spotting caused by the sterile fungus filtrates.

As seen from Table 5, the optimum concentration of dextrose in nutrient solution for toxin production is 1%. As the dextrose concentration was increased from 0.5 to 1%, toxin production as well as mycelium pro-

TABLE 5.—*Relation of dextrose concentration, in nutrient broth, to the mycelium and toxin production of Botrytis allii*

Dextrose conc.	Dry wt. mycelium produced (mg)	Av. no. of spots per plant [a]	Final pH [b]
0.00	30	0	7.25
0.05	50	4	7.12
0.10	66	4	4.88
0.50	135	52	4.95
1.00	246	235	4.61
2.00	302	122	4.20

[a] 5 plants per treatment.
[b] Original pH of media 6.2.

TABLE 6.—*Spots per onion leaf after six weekly applications of fungicides to field plots at Pine Island, New York, 1951* [a]

Spray treatment	Conc. per 100 gal.	Av. no. spots per leaf			
		Rep. 1	Rep. 2	Rep. 3	Av.
Check		198	168	138	168
Dithane Z-78	2 lbs.	118	122	78	108* [b]
Copper A	3 lbs.	153	222	134	170
PEPS	¾ pt.	196	186	160	181
LSD 19:1 = 36.56					
LSD 99:1 = 55.38					

[a] Each spray plot consisted of 8 rows 525 ft. long. Fungicides were applied at a pressure of 110 lb. at the rate of 100 gal. per acre.

[b] Single asterisk indicates that difference between indicated figure and that of unsprayed check is significant at 5% level.

duction increased. At 2% dextrose concentration, toxin production decreased while mycelium growth continued to increase.

Unlike the action of *Botrytis* spores, where light is required for spotting on onion leaves, the toxin from fungus nutrient-solution-filtrates caused spotting equally well whether plants were placed in light or dark following its application.

Rubbing onion leaves with 400-mesh carborundum increased their susceptibility to the toxin. Plants so injured and sprayed with the fungus filtrate had an average of 225 spots, whereas unrubbed plants similarly sprayed had an average of 79 spots. Injuring leaves, before inoculation with a spore suspension of *B. allii*, however, did not increase the amount of spotting. Thus, injuring the leaves increased susceptibility to the action of the toxin but not to the action of the germinating spores.

Field control of blast.—Control studies were conducted in 1951 and 1952 in Orange County, New York, to determine what fungicides would best control the disease. In 1951, Dithane Z-78 (65% zineb, zinc ethylenebis[dithiocarbamate]), copper oxychloride (45% Cu), and PEPS (polyethylene polysulfide) were applied at the concentrations shown in Table 6, in replicated field plots. Triton B 1956, a spreader, was added. Six applications were made at weekly intervals at about 100 gal. per acre. Following the sixth application of fungicides, leaf-spot counts were made using the third youngest leaf from each of four randomly selected plants in each of the three replicates. Results are in Table 6. Zineb significantly reduced the number of spots per leaf as compared with the check plots and all the other treatments.

Extensive fungicide tests were conducted in 1952, but the disease did not appear that year except in one location in Orange County. The results are shown in Table 7. Here again, the number of spots per leaf was recorded as in the 1951 experiments. Fermate (76% ferbam, ferric dimethyldithiocarbamate), captan (50% *N*-trichloromethylthiotetrahydrophthalimide) and Parzate (19% nabam, disodium ethylenebis[dithiocarbamate]) plus zinc sulfate, all significantly reduced the number of spots as compared with those in the unsprayed check plots.

These results are in agreement with those of Newhall and Rawlins (6), who found in 1952 that nabam or zineb on onions gave a high degree of control of blast in several other sections of New York and resulted in a mean increase in yield on 16 farms of 167 bushels to the acre (26%).

Discussion.—It was supposed, as a result of previous investigations, that blast of onions was caused by unfavorable environment, because no organisms had been found associated with the field symptoms of the disease.

The senior author found that the leaf spot phase of the disease could be produced when onion leaves were inoculated with a spore suspension of *Botrytis allii*, *B. cinerea*, or other species of *Botrytis*. This leaf spotting occurred only if inoculated plants were exposed to daylight or strong artificial light following inoculation. Why light is necessary was not determined. An abundance of inoculum was found in onion fields where blast was present in the form of *Botrytis allii* conidia and conidiophores present on necrotic leaves and bulbs. An additional demonstration that blast is of pathogenic origin is seen in the success that Newhall and Rawlins (6) and the senior author had in controlling serious outbreaks of the disease with weekly applications of nabam.

No evidence could be found of penetration by germ tubes of *Botrytis* spores through stomata or the cuticle of onion leaves. This lack of penetration may be why the causal organism could not be isolated from tissue showing leaf spot symptoms. If leaf tissue was surface-sterilized, the disinfectant used would kill the germinating spores on the leaf surface. If leaf sections were placed on artificial culture media without surface sterilization, saprophytic bacteria and fungi present on the leaves would overgrow the *Botrytis*. It is possible that slight penetration does occur (though not demonstrable by the histological methods used), but it seems certain that any growth of fungus hyphae within the tissue would have been detected. Whether penetration

TABLE 7.—*Spots per onion leaf sprayed with fungicides, Pine Island, New York, 1952* [a]

Treatment	Conc. per 100 gal.	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Av.
Fermate	2 lb.	51	116	115	123	101** [b]
Check		166	261	214	329	242
Orthocide 406	2 lb.	34	93	100	63	72**
Check		210	240	247	339	259
Liquid Parzate	2 qt.	72	52	71	53	62**
Check		191	276	354	257	269

[a] Total of 7 applications were made between June 19 and August 12. The fungicides were applied at 150 lb. pressure at a rate of 100 gal. per acre. Each spray plot consisted of 16 rows, 16 in. apart, 525 ft. long. Two untreated check plots, 35 ft. long, extended across the width of the field.

[b] Double asterisks indicate that difference between indicated figure and that of corresponding check is significant at 1% level.

occurs or not, many cells that are not invaded by the hyphae are killed. These uninvaded cells are probably killed by the action of a toxin secreted by germinating spores of *Botrytis*.

When a sterile filtrate of a nutrient dextrose broth culture of *B. allii* was sprayed on onion leaves, it caused small lesions identical to those occurring on plants naturally infected in the field or those artificially inoculated in the greenhouse, or field, with a spore suspension of the fungus. This strongly indicates that the symptoms associated with this disease are the result of penetration of the leaf tissue by a toxic, exogenous substance elaborated by germinating spores of *B. allii*, though the fungus itself may not penetrate the tissue. Applying a protective fungicide to prevent spore germination has given excellent control.—Department of Plant Pathology, Cornell University, Ithaca, New York.

LITERATURE CITED

1. Clinton, G. B. 1904. Report of the botanist. Conn. Univ. Storrs Agr. Expt. Sta. Ann. Rept. (1903) **27**: 279-370.
2. Clinton, G. B. 1914. Report of the station botanist, 1913. Conn. Univ. Storrs Agr. Expt. Sta. Ann. Rept. **38**: 4.
3. Doran, W. L., and Bourne, A. I. 1931. Onion spraying and dusting experiments. Mass. Agr. Expt. Sta. Bull. **279**: 176-185.
4. Jones, L. H. 1944. Relation of weather conditions to onion blast. Plant Physiol. **19**: 139-147.
5. Munn, M. T. 1917. Neck rot disease of onions. N. Y. State Agr. Expt. Sta. Geneva, N. Y. Bull. **437**: 363-455.
6. Newhall, A. G., and Rawlins, W. A. 1952. Control of onion blast and mildew with carbamates. Phytopathology **42**: 212-214.
7. Walker, J. C. 1925. Two undescribed species of Botrytis associated with the neck rot of onion bulbs. Phytopathology **15**: 708-711.
8. Whetzel, H. H. 1904. Onion blight. New York (Cornell) Agr. Expt. Sta. Bull. 218, p. 138-161.

REPORT AND ABSTRACTS OF THE 1959 ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 41st meeting of the Pacific Division of the American Phytopathological Society was held in conjunction with the 40th meeting of the American Association for the Advancement of Science at San Diego State College, San Diego, California, June 17, 18, and 19, 1959.

Officers elected for 1959–60 at the business meeting, held June 17, were: *President*, K. F. Baker; *Vice-President*, J. D. Menzies; *Councilor for the Division*, B. R. Houston. The present *Secretary-Treasurer* was retained for another year.

William A. Kreutzer.

Activity of the antibiotic produced by Pullularia pullulans. Baigent, Nancy L., and Joseph M. Ogawa. *Pullularia pullulans* strongly inhibits the growth of *Sclerotinia fructicola, S. laxa, Botrytis cinerea, Verticillium albo-atrum, Colletotrichum lini, Physalospora obtusa, Cytospora rubescens*, and *Penicillium* spp. in dual cultures on PDA (potato-dextrose agar). Antibiotic production increases with time, as measured by the inhibition of *S. fructicola* by mycelium-free PDA plugs cut from the periphery of replicate *Pullularia* colonies of various ages up to 8 days. The inhibitory substance is produced, and is active, on PDA of pH 3.7, 6.6, 7.2, and 8.6. Production of the antibiotic in potato-dextrose-broth shake cultures is increased by the addition of 1.3% galactose. The crude antibiotic, in the form of a sterile filtrate of a continuously-shaken 8-day-old potato-dextrose broth culture, does not lose activity upon heating at 100°C for 10 hours. Storage at 8°C for 15 days results in no discernible loss of activity. The growth-inhibiting substance is adsorbed strongly from the culture filtrate onto activated charcoal. A hundred-fold dilution of the crude antibiotic, applied with filter-paper bioassay discs, was no longer active in inhibiting the growth of *S. fructicola*.

Aerated steam for soil treatment. Baker, Kenneth F., and Carl M. Olsen. The standard recommendation for steaming soil to free it of pathogens has been to maintain 212°F for 30 min. Although it is recognized that moist heat of 140°F for 30 min. will kill pathogens, a feasible method for thus steaming soil has not been available. Steam temperature may be lowered to any desired level by dilution with air, heated or unheated; the ratio of air/steam at 212°F is 0/1 by weight or 0/1 by volume, at 180° is 1.5/1 or 1.0/1, at 140° is 6.5/1 or 4.1/1, respectively. The manner, rate, and distance of movement of aerated steam through the soil approximates that of pure steam. Aerated steam may be used for treatment of soil by surface or subsurface application, or in a steam vault or some steamers with a moving soil mass. Aeration may be effected by a steam jet exhauster (Venturi) or a squirrel-cage blower. The use of low-temperature steam is desirable because: 1) application cost is reduced; 2) a smaller boiler is required for a given job, or a larger area is treated in a given time; 3) post-steaming soil toxicity is decreased; 4) a portion of the established nonpathogenic soil microflora remains and luxuriates following treatment, providing competition for any pathogens that may be introduced.

Susceptibility of Mazzard Seedlings to Pseudomonas Syringae. Cameron, H. Ronald. Budding or grafting commercial cherry varieties onto scaffold limbs of 2–3-year-old mazzard seedlings has been recommended to reduce tree losses from bacterial canker . Sixty-three trees budded high on mazzard or mahaleb were compared with 61 low-budded trees. Ratings of natural infection from 0 (no canker) to 5 (tree killed by bacterial canker) were used. A rating above 2 indicated cankers on main scaffold limbs. Trees budded high on mahaleb averaged 0.7, budded high on mazzard 1.3, and budded low on mazzard 3.0. Eleven low-budded trees were killed. Inoculation of 250 3-year-old mazzard seedlings with a known culture of *Pseudomonas syringae* gave 100% infection. Some difference in size of canker was noted in trees of different parentage. Cankers varied from 3 in. to 4 ft. in length after 4 weeks. Gum was seldom extruded, and only one tree died. Bing inoculated at the same time had large open cankers and gummed profusely. Infected mazzards showed a faster regeneration of callus tissue than did Bing. Mazzard seedlings are tolerant of but not resistant to bacterial canker.

Electrophoresis of tobacco mosaic nucleoprotein and nucleic acid virus on agar gels. Cochran, G. W., G.W. Welkie, and J. L. Chidester. More than 50 agar electrophore-

sis experiments with varying sources of tobacco mosaic virus have been completed using a sectional plastic U tube apparatus with o-ring-sealed joints. Two 1½-in. removable sections near the base of one arm are filled with buffered liquid agar while set on molds. After gelling and assembly, a 1-mm sample space is left between the agar-filled sections. Standardized conditions of agar, 1%; buffer, pH 4.4—.01*M* phosphate; field strength, 5 volts per cm; current, 1.4 ma per cm^2; time, 10 minutes; water-bath temperature, 2°C, gave the following results. Centrifuge-purified nucleoprotein virus did not migrate. Phenol-prepared nucleic acid virus migrated from 0 to 2.5 cm toward the anode. Leaves grown on systemically infected plants, ground with dry ice, pressed into frozen wafers, and inserted in the sample space, yielded 1% migrating, 99% nonmigrating virus. A circular disc cut from a similar leaf, quick-frozen in ether-dry ice and inserted in the apparatus, yielded 13% migrating, 87% nonmigrating virus. Migration distance and virus titer were determined by local-lesion assay of serially cut agar slices. The migrating virus from the infected tissue was assumed to be a nucleic acid.

The fungicidal activity and sorption of nabam in soil. Corden, Malcolm E., and Roy A. Young. Nabam was highly fungicidal when mixed into soil, but was retained in the surface layers when applied as a drench. Assays were made in a sandy-loam soil artificially infested with chlamydospores of *Fusarium oxysporum* f. *cubense*. Fungicide treatments were made in sufficient water to bring the air-dry soil to field capacity (25%), and chemical concentrations were figured as mg of active chemical per kg of air-dry soil (ppm). Treatment effects were evaluated by soil dilution techniques. Nabam mixed into soil had an ED_{50} of 6 ppm, but drenched onto soil and assayed at 5–6 cm below the surface the ED_{50} was 600 ppm. Thus, under these conditions about 99% of the nabam was retained in the upper 5 cm of soil. Mixed into soil, nabam was more toxic than the structurally related carbamates maneb (ED_{50} 18 ppm) and zineb (ED_{50} 86 ppm), and slightly more toxic than the soil fungicide vapam (ED_{50} 9 ppm). Nabam has been recommended as a soil drench primarily because of its high fungitoxicity and water solubility. However, due to its sorption in the upper layers of soil, nabam should be more effective as a soil mix than as a drench treatment.

Symptoms of Ditylenchus destructor, the potato rot nematode infection, and their development during storage. Dallimore, C. E. No foliage symptoms of *Ditylenchus destructor* are apparent, since infection is restricted primarily to the tuber. The nematode may be present in the tuber for some time before external symptoms appear. A discoloration beneath the periderm is the earliest external symptom and, because of the softness of the tissue, may frequently be detected by the fingers before being seen. With an increase in size and coalescence of lesions, the periderm develops a papery texture, becomes depressed, and eventually breaks, exposing the necrotic potato tissue beneath. The earliest internal symptoms are small pearly white spots with a small hole in the center. On enlarging, the lesions take on a somewhat honeycombed appearance. Eventually these areas develop into gray to black loose woolly lesions that become apparent externally as dark spots beneath the periderm. In advanced stages the remaining portion of the potato tissue and nematode colony take on the appearance of brown matted wool. These areas are soft and spongy. Since these symptoms are not specific for this disease the presence of the nematode is essential for final diagnosis. All stages of infection may be found at harvest. There may or may not be further development of symptoms during storage.

A two-bottom two-way plow sole fumigator. Dallimore, C. E. Under Idaho conditions plow-sole application of soil fumigants for nematode control is usually preferable to chisel application. A two-bottom two-way plow, International No. 39 rollover plow, is readily adapted to soil fumigation. A tractor-power take-off pump of the nylon roller type is used to power the system. Neoprene-lined hoses are used for the suction, supply, and by-pass lines. The control block is made up of 2 by-pass valves (two valves have been found necessary in applying small quantities of fumigant), a quick shut-off valve, a pressure gauge, and brass pipe fittings. Spray nozzle parts and necessary adapters are used for orifice and screen containers. These containers are fastened to pieces of strap iron attached to the plow frame. The stream of fumigant is directed into the plow furrow ahead of the overturning soil. Two outlets per plow share are used with plows over 12 in. in size. For experimental work where different fumigants or different rates necessitate more than one size of orifice, rapid change can be made by attaching several orifices to the applicator and connecting the proper orifice to the delivery tube.

Effect of stem girdling of citrus seedlings on size of Phytophthora gummosis lesions. Dimitman, J. E., and L. J. Klotz. An attempt to alter the resistance of lemon and sweet and sour orange seedlings was made by a series of girdling experiments. Four seedlings of each species, ¼–½ in. in diameter, were planted to each 5-gal. can. They were topped to uniform height, and 2 months later 1 tree per can was ringed by removing a ¼-in. strip of bark completely around the trunk and lightly scraping the wood beneath. One week after the first series, another tree was ringed in each can, and on the third week a third tree was also ringed. On the fourth week all 4 trees, including the one not girdled, were inoculated about 1½ in. above and 1½ in. below the girdle with an isolate of *Phytophthora citrophthora* (2104) and an isolate of *P. parasitica* (55127B). The trees were placed on benches in the glasshouse, where the maximum temperatures remained below 32°C for 60 days. The cankers were scraped and margins delimited and traced. The split-plot analysis of 144 trees showed that girdling in the sweet orange reduced lesion size, especially below the girdle. This was also the case with lemon, but less so. In sour orange only slight differences were noted. The lesion size below the girdle was reduced, in general, in all 3 *Citrus* spp.

Vegetative growth of Phytophthora spp. on differential synthetic media as an aid in separating isolates pathogenic to citrus. Dimitman, J. E., and G. A. Zentmyer. Mycelial dry weight was used as a basis in studies on the carbon-nitrogen nutrition of *Phytophthora* spp. It was found that there were differences in the ability of *P. parasitica* and *P. citrophthora* to utilize different amino-acid–carbohydrate combinations. Certain amino-acid–carbohydrate combinations produced excellent growth with both species, other combinations inhibited one or the other species. It is felt that differential media can be utilized to aid in the differentiation of these and other *Phytophthora* spp. pathogenic to citrus. A medium suggested for the identification of *P. citrophthora* would contain maltose or dextrin as the carbon source with l-asparagine as the nitrogen source. *P. parasitica* on this combination, along with salts and vitamins, produces less than one-seventh the growth produced by *P. citrophthora*. When a monosaccharide, such as glucose or fructose with l-glutamine or l-glutamic acid, was used in the basal medium, excellent growth was obtained for *P. parasitica*, whereas *P. citrophthora* grew very sparsely. Other *Phytophthora* spp. apparently fall into one of the three categories: those growing on either but not both amino acids, and those growing on both amino acids; and linear growth on solid synthetic media can be used as an aid in identifying them.

Control of citrus fruit decay with dichloroisocyanuric acid. Eckert, J. W., M. J. Kolbezen, F. O. Whipple, B. F. Loehr, and R. J. Fuchs. Paper sheets impregnated with sodium dichloroisocyanurate (NaDCCA) reduced decay in oranges artificially inoculated with *Penicillium digitatum*. Sheets (11×17 in.) were impregnated with 0.41 g, 0.96 g, 2.20 g, or 3.12 g NaDCCA. Two equal-concentration sheets were placed in each fiberboard fruit carton. Inocu-

lated fruit were evaluated for decay and fungus sporulation at the end of a 15-day simulated market-handling period. All NaDCCA treatment levels reduced decay more than 50% below that of untreated control fruit. In addition, treatment with NaDCCA at 2.20 g or 3.12 g per sheet greatly suppressed fungus sporulation on the surface of decayed fruit. The evolution rate of chlorinous volatile material from moistened NaDCCA-impregnated sheets is accelerated by ammonium salts. Combination formulations, consisting of paired NaDCCA and $(NH_4)_2SO_4$ sheets, provided more effective decay control than NaDCCA sheets alone. This combination usually produced fruit injury and did not control sporulation over the 15-day test period. Vapor-phase spectrophotometry and chemical analysis of trapped effluents indicate that NCl_3 is the principle volatile material arising from moistened NaDCCA-impregnated sheets.

Effect of a virus on the winter-killing of oats. Endo, R. M., and C. M. Brown. In Illinois, the effect of yellow dwarf on winter oat varieties Lee, Dubois, and Wintok, and Hudson winter barley was measured by comparing the winter survival and yield of diseased and healthy plants. Field plots were infested with viruliferous apple-grain aphids, *Rhopalosiphum fitchii*, on October 21, November 7, or November 20; the check plots were not infested. These aphids were reared in the greenhouse on caged Hudson barley plants infected with a moderately virulent strain of the barley yellow dwarf virus (Illinois I). Symptoms did not develop until April, 1958, when the percentage differences in winter-killing between inoculated and uninoculated varieties for the 1, 2, and 3 dates of inoculation, respectively, were (significant figures shown by asterisk): Wintok 31.7*, 10.0*, 6.7; Dubois 36.7*, 8.4*, 3.4; Lee 40.0*, 10.7*, 6.7; Hudson 30.0*, 21.7*, 3.3. Average infection in the plants that survived the winter was 8.3% for the uninoculated checks, and 99.1, 99.8, and 97% for plants inoculated on the 1, 2, and 3 dates, respectively. Reduced yields following inoculation on the 3 dates were: Dubois, 95.7, 68.6, 65.5%; Lee, 93.4, 85.0, 72.5%; Wintok, 61.4, 56.1, 57.3%; Hudson, 96.6, 76.0, 57.6%.

Variation in isolates of Pseudomonas associated with blast and canker of fruit trees in California. English, Harley, and James R. Davis. Green-fluorescent *Pseudomonads* isolated from stone fruits, pears, and citrus have been found to vary considerably in cultural characteristics, physiology, and pathogenicity. When grown on an agar medium containing proteose peptone as the nitrogen source and glycerol as the carbon source, the following broad colony types were observed: 1) translucent greenish-white, flat or with a slightly raised margin; 2) yellowish green, opaque, convex, and slimy; 3) bluish-gray, opaque, raised. All three types produced a water-soluble greenish-yellow pigment (fluorescein) on this medium, but on potato-dextrose agar only the second type formed this pigment. Of the pathogenic isolates tested, only those of type 2 formed acid in purple lactose agar. Type 2 also showed weak pectolytic enzyme production, whereas type 1 lacked this capacity. Type 1 generally caused larger cankers on stone fruit trees than the other forms. Type 2 was much more pathogenic to green pear fruits than were the other types, and it was generally more pathogenic to bean stems. No means have been found for distinguishing between pathogenic and nonpathogenic green-fluorescent Pseudomonads other than by inoculation of living plant tissue. Type 1 isolates fit nicely within *Ps. syringae*, but the concept of this species would need to be broadened somewhat if it is to include the other two types.

A tobacco-necrosis-like virus isolated from potato-tuber lesions and California soils. Gold, A. Herbert. A tobacco-necrosis-like virus has been isolated from lesions on potato tubers grown in sandy soils of the Salinas Valley in California. The virus could not be isolated from symptomless areas on the same tubers. These tuber lesions, while milder, resemble those reported by Noordam, in Holland. When soil samples from the fields where these potatoes were grown was extracted with 0.05*M* K_2HPO_4 and the extracts inoculated to Pinto bean, Beka bean, or cowpea, characteristic lesions were produced on the inoculated leaves. Similar lesions resulted from inoculations of extracts of other sandy soils and from uncultivated coastal dune sand, but not from heavier soils. Electron microscopy indicates the virus to be a small spherical particle.

Morphology and host range of a subterranean member of the Meliolaceae. Gordon, C. C., C. G. Shaw, and J. D. Menzies. This undescribed species of the *Meliolaceae* causes a disease referred to as "black root" of potato (*Solanum tuberosum* L.) and egg plant (*S. melongena* L.) on irrigated land in the Columbia Basin. The fungus produces a dense, superficial reticulum of dark mycelium on the fibrous root system. Penetration is effected by an infection peg produced by the terminal cell of a two-celled, capitate hyphopodium. The hyaline, bulbous haustoria are confined to the epidermis. One to several haustoria may be formed in a single epidermal cell. The perithecia are flask-shaped and ostiolate, with the perithecial wall 2–4 cells thick. Forty-four tuber-forming and 8 non-tuber-forming species of *Solanum* proved susceptible in field and greenhouse tests. Potato and egg plant are the only economically important crops parasitized. Species belonging to 11 other genera of the Solanaceae have shown no infection. The pathogen has not been observed on native and cultivated species belonging to other families of flowering plants.

The amount of Verticillium albo-atrum in Idaho certified potato seed. Guthrie, James W. *Verticillium albo-atrum* has been shown to be carried in potato tubers. In an effort to determine the percentages of tubers grown in the certified potato areas that carry this organism, isolations were made from about 1800 tubers from 8 localities. The tubers were surface-sterilized, and a wedge of tissue about 4 mm deep was removed from the stem end and placed on PDA in Petri dishes. All fungal cultures issuing from the tuber wedge were examined. Those suspected of being *V. albo-atrum* were subcultured for more definite identifications. The percentage of tubers from each area infected with *V. albo-atrum* was as follows: 0.0 in Teton Basin and McCall, 0.2 in Grace-Soda Springs, 0.4 in Ashton-Lost River-Miscellaneous and 2.7 in North Idaho. As a check on technique, isolations were made from potatoes grown in the Aberdeen and Rexburg areas, in both of which Verticillium wilt is endemic. The per cent of tubers from which *V. albo-atrum* was obtained was 16.3 for Aberdeen and 30.0 for Rexburg.

A holly leaf spot associated with the use of copper fungicides. Herridge, E. Anne, and R. C. Lambe. Copper compounds, in particular tribasic copper sulfate, have been tested and used extensively in Oregon for control of green algae and Phytophthora leaf and twig blight on English holly. Because of reports of injury following use of copper sprays, additional tests were conducted in 1958 in which tribasic copper sulfate, at rates of 2, 4, and 8 lb./100 gallons of water and copper sulfate at 2 lb./100 gallons were applied to young trees. These applications were made in April and June. By midwinter, raised, circular, red spots were apparent on the under surface of sprayed leaves. Severity of spotting increased with increased rates of chemical. The spots apparently centered on stomata, and anatomical investigations showed deposition of resinous substances in the substomatal cells. The spongy mesophyll was greatly proliferated, and, through hypertrophy of the cells, the intercellular spaces were obliterated. Enlarged cells showed little or no chlorophyll. Although trials have been conducted on trees of different varieties and sizes for the past 5 years, this is the first instance in a controlled spray trial where injury has been associated with the use of copper.

Occurrence of Cytospora canker in stone fruit trees in California. Lukezic, F. L., J. E. DeVay, and H. English.

Isolates of Cytospora (tentatively identified as *C. rubescens* and *C. leucostoma*) have been implicated in the complex of organisms that cause cankers on stone fruit trees in California. The incidence of Cytospora cankers on *Prunus domestica* (var. President and Imperial) has been most evident; however, these cankers have also been observed on *P. amygdalis* (var. Texas) and *P. avium* (var. Bing). Cankers caused by *Cytospora* are perennial and continue their development during the growing season, in contrast to the more common bacterial cankers caused by *Pseudomonas syringae*. Invasion of healthy tissues by *Cytospora* has been observed only in cases where bark injuries or dead tissues have served as infection courts. Healthy trees that are near trees severely infested with the shot-hole borer, *Scolytus rugulosus*, are often attacked and weakened; the injured and weakened tissues apparently serve as avenues of entrance for *Cytospora*, which has been frequently isolated from necrotic tissues surrounding the bark tunnels. Injured or dead plant tissues are favorable substrates for the growth of *Cytospora*, apparently because of the greater availability of certain growth factors in weakened tissues. All isolates of *Cytospora* studied thus far have multiple biochemical deficiencies. Growth equal to that on potato-dextrose broth has been obtained by growing the isolates of *Cytospora* in a sugar-salt solution containing a mixture of 10 water-soluble vitamins. Possible relationships between the growth-factor requirements and the pathogenicity of *Cytospora* are discussed.

The diploid nucleus of Puccinia carthami in unstained water mounts. McCain, Arthur H. The diploid resting nucleus in each cell of the two-celled teliospore of *Puccinia carthami* is plainly visible in unstained water mounts. It appears as a large sphere 7.5–7.7 μ in diameter, located in the center of each cell. A single nucleolus is evident when the nucleus is stained with hematoxylin or phloxine-fast green. When spores are centrifuged the teliospore nuclei sink, i.e., move in the direction of the force, while fat globules in the urediospores rise. In a matter of minutes the fat globules become redistributed. Several hours are necessary for the teliospore nucleus to return to the center of the cell. It remains dislocated for at least 3 months in centrifuged spores that have been dried rapidly. The mild centrifugation, 1400 G for 15 minutes, used to dislocate the nucleus did not prevent germination of the teliospores. A prominent spherical object, probably the nucleus, is visible in water mounts of teliospores of *P. graminis*, *P. malvacearum*, and *P. asparagi*.

Symptoms of Verticillium wilt of castor bean. Mueller, Kenneth E., and Byron R. Houston. During a study of factors involved in the capsule blight of castor bean (*Ricinus communis*) in the Sacramento Valley of California it was found that *Verticillium albo-atrum* was commonly isolated from roots, stems, petioles, and racemes of field-grown plants. Seedlings of the variety U.S. 3/384-8-6 two weeks of age were inoculated by dipping roots in a *Verticillium* spore suspension and transplanting. The cotyledons of such plants wilted rapidly 13–20 days after inoculation. Small areas of veinal discoloration occurred first on leaves, followed by chlorosis and necrosis of interveinal areas. As the plant elongated, the fungus moved upward but remained 1–3 nodes behind the growing point. Newly developed leaves often showed interveinal chlorosis. Sections and isolations indicated that the fungus was not present in such leaves. After raceme formation the fungus moved into the racemes, resulting in necrosis of branches and blighting of capsules. Twenty days after inoculation, many small overgrowth areas appeared on the hypocotyl, and similar overgrowths later occurred on the stems. This roughening of the stems was due to abnormal increase in cell size and numbers in the cortex adjacent to invaded and damaged vascular bundles. Small islands of cell necrosis developed in the cortex.

Juice transmission of cucumber mosaic virus to mazzard and mahaleb cherry. Nyland, George. A virus obtained from ringspot-free Shiro plum showed certain similarities to CMV (cucumber mosaic virus). Inoculation with expressed juice from leaves of *Zinnia elegans* and *Nicotiana glutinosa* infected with CMV originally obtained from cantaloupe in Imperial County, California, produced symptoms in mahaleb and mazzard cherry but not in almond or peach seedlings. Symptoms were much more abundant and obvious in mahaleb than in mazzard, although the virus was readily recovered in diagnostic herbaceous hosts from both. The virus was transmitted from infected mahaleb to mahaleb. In mahaleb, vein-clearing appeared in inoculated leaves after 7 days and progressively developed into yellow-net and veinbanding by the 14th day. Some inoculated leaves showed a chlorotic spot mottle. Some leaves one node above those inoculated also developed symptoms after 15–20 days. In mazzard, symptoms were limited to vein clearing and eventual veinbanding, but without yellow color. Six additional strains of CMV were juice-inoculated into mahaleb seedlings at the 3-leaf stage. Five strains produced vein-clearing, netting, veinbanding, and spot mottle, but 1 strain failed to infect mahaleb seedlings. CMV was readily recovered from plants infected with the 5 strains.

Relations of spore moisture content to spore shape and germination reaction to temperature. Ogawa, Joseph M., and Arthur H. McCain. Wet spores shrivel rapidly upon drying. Shriveled spores retain their features in immersion oil. Spores of *Sclerotinia fructicola*, *S. laxa*, *Pseudoperonospora humuli*, *Puccinia carthami*, *P. cyani*, *Ustilago hordei*, *Coryneum beijerinckii*, *Penicillium digitatum*, *Botrytis cinerea*, *Rhizopus stolonifer*, and a *Mucor* sp. were found on their hosts in the shriveled condition. Shriveled spores are drastically different from wet spores in shape. Exposure of wet and dry spores to various temperatures for 10 minutes resulted in a sharp drop in germination near the thermal death point. Thermal death range, in °C, was as follows: *S. fructicola*, wet 40–45, dry 90–95; *S. laxa*, wet 40–45, dry 80–85; *U. hordei*, wet 45–50, dry 90–95. Dry spores of the following fungi on glass rods were killed when exposed 1 second to live steam at 100°C: *S. fructicola*, *R. stolonifer*, a *Mucor* sp., *P. digitatum*, *P. italicum*, *P. expansum*, *Cladosporium herbarum*, *B. cinerea*, and *Aspergillus niger*.

A Fusarium wilt of Tagetes. Olsen, Carl M. A vascular Fusarium wilt caused heavy losses of African marigold (*Tagetes erecta*) in southern California seed fields during October, 1957. Pathogenicity of the *Fusarium oxysporum* consistently isolated from these plants was shown with single-spore cultures in steamed soil. In these tests a 97% loss occurred in the original field line, but many other commercial varieties were resistant. Mycelium extended to within a few cm of the plant apex. Seed transmission (2–10%) occurred. Most commercial varieties of China aster were highly susceptible to the aster and Tagetes forms, expressing similar symptoms. *T. erecta* was highly susceptible to the Tagetes Fusarium but not to the aster form. A reselection of Ball White aster, 99% resistant to aster wilt, and the French marigold (*T. patula*) were highly resistant to the Tagetes form. Dahlia, Shasta daisy, English daisy, Calendula, Dimorphotheca, Gaillardia, and zinnia were immune. Centaurea Double Blue Boy was about 45% susceptible. Pathogenicity to Tagetes was retained following passage through aster or Centaurea, or growth in culture for at least 1½ years. Cultures of the aster wilt and Tagetes wilt organisms are morphologically similar.

A root disease of fuchsia caused by Phytophthora parasitica. Patil, S. S., and Roy A. Young. In the summer of 1958 a severe outbreak of fuchsia root rot occurred in a greenhouse and lathhouse near Portland, Oregon. Affected plants wilted suddenly and died within a few days. *Phytophthora parasitica* was cultured consistently from roots and stems of dying plants and was shown by inoculation tests to be the cause of the disease. The disease developed in plants inoculated and incubated at 85°F, but not at 65° or 75°F. Infection occurred at 75°F, but disease symptoms

did not develop unless plants were moved to higher temperatures. Plants inoculated and incubated at 85°F for 24 and 48 hours and then moved to 75°F became infected but did not develop symptoms. Plants inoculated and incubated at 85°F for 80 hours developed symptoms and died even though transferred to 75°F. Mean summer temperatures in June, July, and August, 1958, were about 5°F above mean temperatures for 1955, 1956, and 1957. Temperatures above 90°F were recorded on 15 days in 1958, as compared to 5, 3, and 0 days, respectively, in the other years. The outbreak of fuchsia root rot in 1958 is attributed to latent infections and summer temperatures higher than normal.

Relations between inoculum density and infection of wheat by uredospores of Puccinia graminis var. tritici. PETERSEN, LARRY J. Increasing attention has been given to the determination of actual numbers of infective units placed on a host plant. Accordingly, in this study, uniform reproducible inoculations were accomplished by means of a spore settling tower. Infection was facilitated by maintaining a temperature of 74 ± 1.5°F and 100% RH in an incubation chamber constructed at the base of the tower. A pure culture of physiologic race 15-B was used to inoculate Little Club wheat seedlings. Direct counts were made of uredospores, germ tubes, appressoria, infection foci, chloronemic flecks, and uredia per cm^2 of leaf surface. As inoculum density was increased the number of uredia per cm^2 of leaf surface increased linearly to a maximum of .7 uredia per 100 viable uredospores. There was a distinct tendency for the type 4 reaction class, observed at low inoculum densities, to assume the characteristics of less susceptible infection types at high inoculum densities. This conclusion was based primarily on a size-of-pustule relationship, since the difference in degree of chloronemia was not great between inoculum densities. The infection response curve, although linear, was somewhat bimodal in the following respect: at inoculum densities between 2 and 4 × 10^3 viable uredospores per cm^2, very little difference in number of uredia occurred, whereas the number of uredia increased 2-fold at inoculum densities between 4 and 5.2 × 10^3. Further increases in inoculum density resulted in no additional numbers of uredia. The coalescing nature of infection foci was observed, thus explaining in part why uredial counts were much lower than counts of infection foci.

Mutual exclusion of strains of tobacco mosaic virus. SIEGEL, ALBERT. A quantitative study has been made of the number of necrotic lesions induced on leaves of *Nicotiana sylvestris* by a mixture of 2 strains of tobacco mosaic virus. The strains used were U1, which does not induce the necrotic reaction in this host, and U2, which does. A series of inocula were tested that contained the same amount of the U2 strain and different amounts of the U1 strain. It was found that the greater the amount of U1 strain in the inoculum, the fewer were the number of lesions induced. Thus, the presence of U1 strain in the inoculum interferes with lesion induction by the U2 strain. If, however, the leaves are first inoculated with the U2 strain and a short time later, superinoculated with a high concentration of the U1 strain, the number of lesions induced by the U2 strain is not reduced. This indicates that whether an infection results in a lesion is determined at the time of inoculation. The quantitative data are compatible with the notion that only one virus particle can initiate and participate in an infection. When several particles are present at an infectible site, each has an equal probability of being the one that will initiate the infection, regardless of which strain it is a member of.

Some physiologic responses of Glomerella cingulata to various media and growth substances. STROBEL, J. W., AND GRAHAM, S. O. Natural and artificially induced anthracnose infections on foliage plants in Washington are erratic in producing setae on acervulae. Preliminary studies have demonstrated that nutrients affect this phenomenon in part. A setae-producing isolate failed to do so on synthetic medium augmented by ether-acetone extractions of *Dieffenbachia amoena* leaves. Numerous setae were produced on the medium alone or augmented with water extractions of *D. amoena* leaves. A non-setal isolate from *Philodendron panduraeforme* grown concurrently on these same media produced setae on the water extract fraction only. On 10 diverse organic media 8 lines from a single ascus produced setae only on LBA and pea extract agars. However, on synthetic media plus casein hydrolysate, setae were produced. These same lines and 4 monoconidial setal or non-setal isolates from different hosts were cultured on Andes-Keitt medium. Thirteen nutrients and growth factors were added. Choline chloride enhanced growth of all; B_1, B_6 and uracil inhibited growth of all. Monoconidial isolates were stable in setae production, but variability occurred among the ascospore lines. Ascorbic acid and calcium pantothenate prevented setae production. A non-setal line mutant produced setae on all media except that with ascorbic acid.

Relation of age of potato plants to infection by Verticillium albo-atrum. TOLMSOFF, WALTER J. During the past 3 years isolations were made from roots, stems and stolons of several hundred Russet Burbank potato plants grown in field and greenhouse soils heavily infested with *Verticillium albo-atrum*. Under all conditions wilt symptoms were absent until after tubers were set. In 1958, isolations were made from over 7000 linear in. of roots and 1163 in. of stolons taken from 40 plants 28, 38, 48, 61, and 83 days after planting in the field. Root and stolon sections ½ in. long were plated serially. Stolons and tubers were set after 28, but before 38, days. At 28 days localized root invasions were sparse, and systemic invasions were absent. After tuber initiation all roots were susceptible to infection. Frequency of local root infections increased progressively with time to 48–61 days, occurring about 5 times as frequently as at 28 days. At 83 days local root infections were almost entirely absent. Systemic root invasions first appeared after 38 days. Their numbers corresponded closely to the cumulative number of local infections until systemic root invasions coalesced. Stolon infections occurred primarily through small rootlets at stolon nodes, and local infections first appeared after 48 days. Their frequency increased progressively to 83 days.

Pathogenic variation of Cytospora rubescens isolates on stone fruit varieties. TRESHOW, MICHAEL, J. FRED SCHOLES, AND WAYNE S. GARDNER. Isolates of *Cytospora rubescens* were found to be pathogenic in varying degrees to 7 stone fruit varieties inoculated over 2 years. Trees used were of the same age and vigor. Two of the 10 isolates tested were consistently highly virulent and produced 100% infection on vigorous young stone fruit trees regardless of variety or species. Conversely, less virulent isolates were relatively innocuous on most of the varieties. The differences in virulence between isolates proved to be statistically significant. Golden Jubilee and Elberta peach and Fellenberg prune were most susceptible to infection and canker development, and proved to be significantly more susceptible than the more resistant varieties. While clear host specificity was lacking, the significantly greater virulence of generally innocuous isolates on certain varieties suggested that some degree of host preference existed; however, much of the variation in severity of *Cytospora* cankers in commercial orchards is attributed to differences in virulence between variants of the fungus. It is postulated that cankers are most severe in orchards where the more pathogenic variants of the fungus are present, provided that other conditions (age, vigor, etc.) are similar.

Pathogenicity on citrus of Thielaviopsis basicola and its isolation from field roots. TSAO, PETER H., AND S. D. VAN GUNDY. *Thielaviopsis basicola* is commonly found in citrus soils of southern California. Root infection on citrus in the field, however, had not been known. Infestation of soil with this fungus in greenhouse experiments produced marked reduction in the growth of seedlings of Homosassa

sweet orange, Keen sour orange, and Cleopatra mandarin. Many other citrus species, varieties, and hybrids were also found to be susceptible hosts. The severe stunting was the result of a reduction in new root growth caused by infections, mainly on the fibrous roots. Heavily infected roots had a dark appearance. Mycelia and chlamydospores in the well-defined, small lesions were both inter- and intracellular, and were usually confined to the root cortex. Examinations of citrus roots from many orchards revealed typical *Thielaviopsis* lesions, although agar-plating of field roots nearly always failed to yield *T. basicola*. Satisfactory isolation was achieved, however, by inserting surface-sterilized root pieces into narrow slits made on cut surfaces of carrot roots and incubating under moist conditions for 5–7 days. With the "carrot-half" isolation technique, it was shown that field infection of citrus roots by *T. basicola* is common in southern California.

Influence of growing, curing and storage practices on development of neck rot in onions. VAUGHAN, EDWARD K. Excessive nitrogen fertilization and late irrigation resulted in increased incidence of neck rot in Sweet Spanish onions of any given size, and the incidence of disease was proportional to the size of the onions. Curing on the ground for 3–4 days after undercutting but before topping helped prevent neck rot, but the length of the curing period was not critical. Combinations of curing with tops on and additional curing in the field after tops were removed were not better than corresponding periods of curing by either method alone. Drying 24 hours in rapidly moving air at 95, 105, and 115°F resulted in greatly reduced losses from neck rot. Higher temperatures or longer drying periods were injurious to the onions. In all cases more rot developed in onions stored in burlap bags than in onions stored in wooden crates. To ensure a reasonable incidence of neck rot infection in test blocks, spray inoculation using 120 gal./A. of water containing *Botrytis allii* spores applied late in the day proved most satisfactory. An equal amount of water without spores was almost as effective.

Dead button, a non-transmissible disorder of Late Montmorency cherry. WADLEY, BRYCE N. An abnormality, designated here as dead button, was observed in 1949 in several trees, apparently replants of a variety locally known as Late Montmorency, in an orchard of Montmorency cherries near Brigham City, Utah. The same disorder was observed in 1956 in a block of 21 trees of Late Montmorency belonging to the same grower at North Ogden, Utah. Fruits on affected trees may develop necrotic areas at the stylar ends. These necrotic areas, which vary in size, begin development while the fruits are small and green, and progress as the fruits develop. The necrotic area frequently extends to the stone and often separates from the healthy fruit tissue at maturity and leaves a hole in the stylar end of the fruit. The disorder recurs each year on the same trees, but the number of affected fruits varies from year to year. No spread to other trees in the area was noted. Transmission was attempted by budding at various times to Montmorency cherry and to Elberta peach without success.

The effect of host nutrition on the development of exocortis in Poncirus trifoliata. WEATHERS, L. G. The effect of nitrogen and phosphorus on host growth and development of exocortis in *Poncirus trifoliata* rootstocks with Eureka lemon (*Citrus Limon*) tops was studied by growing the host in quartz sand and supplying nutrient solutions varying in nitrogen and phosphorus. In the case of nitrogen, plants showed an optimum growth at 210 ppm. Above that level, marked stunting occurred. Development of exocortis symptoms was directly correlated with the increase of nitrogen. At 1050 ppm, bark symptoms characteristic of exocortis appeared in *P. trifoliata* rootstocks within 10 months of inoculation. At 630 ppm, symptoms developed within 14 months. Symptoms did not appear in plants at the 210 and 50 ppm levels after more than 21 months. With phosphorus, optimum growth occurred at 37 ppm. Some stunting occurred at higher levels, but was much less marked than with nitrogen. Plants at the highest level of phosphorus (547 ppm) developed exocortis symptoms within 14 months of inoculation. Exocortis symptoms did not appear in plants at levels of phosphorus below 547 ppm after more than 21 months. Bark samples were removed from the rootstocks 15 months after inoculation, sectioned, and treated with phloroglucinol-HCl. Plants showing bark symptoms of exocortis all gave positive color reactions; those without symptoms all reacted negatively.

Methods of isolating Phytophthora cinnamomi from soil and from host tissue. ZENTMYER, GEORGE A., J. D. GILPATRICK, AND W. A. THORN. Fuerte avocado fruit provide an excellent trap for *Phytophthora cinnamomi*. Firm fruit are half-embedded in soil in containers, and the soil flooded with water. In 3–6 days infections appear as brown, firm, circular spots at the water line. Other fungi rarely invade firm, unwounded fruit. The optimum period for leaving fruit in the mud trap was 48–96 hours, with some infection in 24 hours. The optimum temperature for fruit infection was 27°C, with light infection at 12° and none above 30°C. *P. cinnamomi* was also recovered when unwounded apples were half-embedded in soil or when holes bored in apples were packed with moist soil, but other fungi interfered with the assay. Placing avocado fruit in wire baskets in the upper part of a soil-water suspension resulted in recovery of the fungus. *Persea indica*, a small-fruited avocado relative, also provided a rapid means of isolating *P. cinnamomi* from soil. Small seedlings developed stripe cankers on the stem, often within 2–3 days of being planted in infested soil. *P. cinnamomi* is readily isolated from diseased avocado trees by placing pieces of small, washed, infected roots, dipped briefly in 70% alcohol and blotted dry, on cornmeal agar plates. In 48 hours at 20–24°C, the fungus is easily identified; later, other fungi overgrow it.

AN ENRICHMENT METHOD FOR ISOLATING ACTINOMYCETES THAT PRODUCE DIFFUSIBLE ANTIFUNGAL ANTIBIOTICS

Peter H. Tsao, Curt Leben, and G. W. Keitt

Respective present addresses: Department of Plant Pathology, University of California, Riverside; Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, Wooster; and Department of Plant Pathology, University of Wisconsin, Madison.

Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

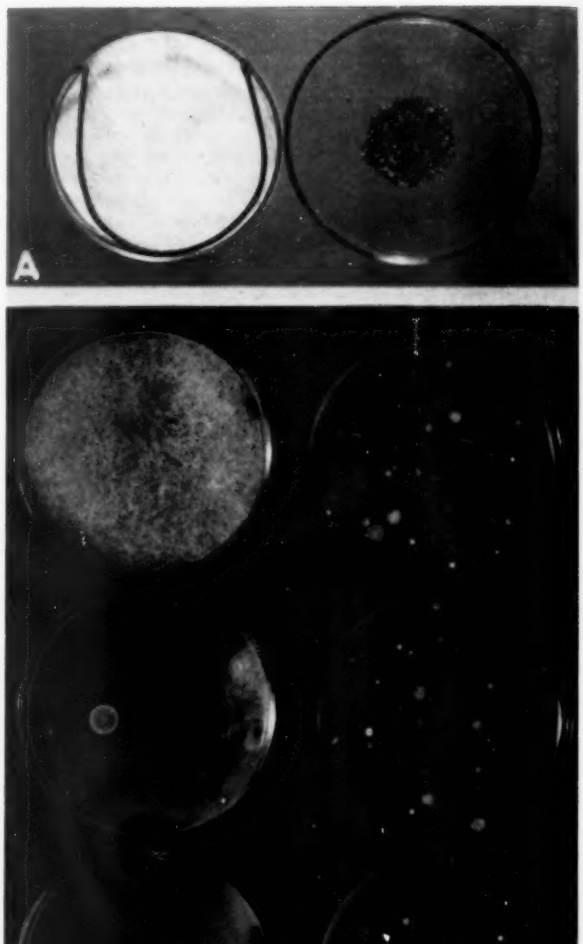
Supported in part by the Research Committee of the Graduate School with funds supplied by the Wisconsin Alumni Research Foundation.

Appreciation is expressed to E. H. Herrling for preparing the photographs.

In previous work (1) a method was described for selecting microorganisms that produce diffusible antibiotics. Agar dilution plates of soil samples were made, and after 7–14 days, the plates were sprayed with a mixed spore suspension of several mutually non-antagonistic plant-pathogenic fungi. Inhibition zones of the fungi around individual colonies of organisms from the soil indicated the production of diffusible antibiotics. The most fruitful group of organisms for antifungal materials was found to be the actinomycetes. Not infrequently, however, fast-growing fungi from the soil overran the actinomycete colonies.

Several years after these first tests, a second series was planned. In view of the previous difficulty with unwanted fungi, it was sought to alter the technique with the aim of reducing the fungi and encouraging the actinomycetes. Selective media that contained antifungal substances and would ordinarily achieve this purpose were not suitable for this work, since they would suppress also the test fungi which were later sprayed on the plates. After numerous preliminary experiments, which are not reported here, it was found that fungi could be reduced by first air-drying the soil and then incubating it moist with $CaCO_3$. The rationale of the procedure was that drying reduced the initial microbial population in the soil, and that the pH of the soil after adding $CaCO_3$ would be changed to favor the growth of the remaining actinomycetes (2) and to inhibit or retard that of most fungi. Thus, with a favorable environment for the growth of actinomycetes, chances would be increased for isolating actinomycete species that occurred in small numbers in the unaltered soil. A given soil sample would then yield a higher number and a greater diversity of isolates, which in turn might yield a diversity of antibiotics. Additional work led to the development of a standardized method which is described below.

Soil samples were pulverized and spread in thin layers in metal trays at room temperature for 5–9 days to dry. They were then stored in glass containers until used. One gram of each sample was mixed with about 0.1 g of powdered $CaCO_3$ and incubated in a closed, inverted Petri plate at 26° C for 7–9 days. High relative humidity was provided by saturating, with sterile distilled water, 8 pieces of filter paper fastened with arched wire in the plate bottom (Fig. 1-A). After incubation, soil suspensions were made with sterile distilled water and incorporated in a 40–45° C soybean meal glucose agar medium[1] that had been adjusted with 1*N* NaOH to pH 7.9–8.1. The final dilution of soil in the medium was made 1:30,000,000.



[1] 17 g of agar per liter were added to the 1 in 8 dilution of the supernatant of an autoclaved liquid medium (per liter: soybean meal, 40 g; glucose, 40 g; $CaCO_3$, 3 g). The pH of the medium was 5.5–5.8.

Fig. 1. A) Plate showing arrangement for moist incubation of soil. Left, plate bottom with moist filter papers held in place by an arched wire; right, inverted plate top containing soil sample mixed with powdered $CaCO_3$. **B)** Comparison of soil dilution plates (1:40,000,000) of an untreated compost soil (3 plates at left) and the same soil that had been dried, mixed with $CaCO_3$, and incubated under moist conditions (3 plates at right). Note the increase of actinomycetes and reduction in fungus colonies in the plates at right. The picture was taken 4 days after plates were poured—before the plates were sprayed with test fungi. Clearings on the medium around some of the colonies were not inhibition zones, but were the result of digestion of solid soybean meal particles.

Fifteen replicate Petri plates, each containing approximately 20 ml of medium, were poured for each soil. Plates were incubated at 26°C. With this method, substantial reduction in the ratio of fungi to actinomycetes was accomplished with most soils (Fig. 1-B). The number of bacterial colonies was not greatly reduced. Most of these, however, did not interfere with the growth and isolation of the actinomycetes. There were usually 10–30 actinomycete colonies per plate. For soils that had unusually low or high population of actinomycetes, lower or higher dilutions, respectively, were employed in a second series of tests to yield a desirable number of actinomycetes per plate.

Five or 6 days after soil dilution plates were poured, most of the actinomycete colonies had grown to macroscopic size. A standardized, mixed, conidial suspension of 5 plant-pathogenic fungi was then sprayed with a compressed air atomizer onto the surface of the agar; each dilution plate was sprayed for about 3 seconds—until the agar surface was wetted. The 5 fungi used were *Alternaria solani* (Ell. & G. Martin) Sor., *Fusarium oxysporum* Schlecht. f. *lycopersici* Sacc., *Glomerella cingulata* (Stonem.) Spauld. & Schrenk, *Helminthosporium victoriae* Meehan & Murphy, and *Verticillium albo-atrum* Reinke & Berth. Preliminary tests showed that the 5 fungi were mutually nonantagonistic and grew well on the isolation medium used. After incubating the plates for 3 more days at 26°C, the actinomycetes that were surrounded by large, clear inhibition zones were isolated. With this altered method approximately 40,000 actinomycete colonies from 81 soils were examined. Two hundred and fourteen that produced antibiotics effective against all 5 fungi were selected and isolated. A majority produced inhibition zones of 25 mm or more in diameter.—Department of Plant Pathology, University of Wisconsin, Madison.

LITERATURE CITED

1. Stessel, G. J., C. Leben, and G. W. Keitt. 1953. Screening tests designed to discover antibiotics suitable for plant disease control. Mycologia **45**: 325-334.
2. Waksman, S. A. 1950. The actinomycetes: their nature, occurrence, activities, and importance. Chronica Botanica Co., Waltham, Mass. 230 p.

ON THE INFECTIVITY DILUTION CURVE OF PLANT VIRUSES

Albert Siegel

In a recent paper, Lindner et al (2) presented a new concept to account for the infectivity dilution curve commonly observed for plant viruses on a local-lesion host. They proposed that the number of lesions observed with high inoculum concentrations is primarily limited by the coincidence of infection of two or more cells within the area that will be occupied by a lesion. This is in contrast to previous proposals (1, 3) that the number of infectible sites is the chief limitation to the number of lesions that can be formed. This note points out that it is unlikely that the coincidence concept applies under the conditions of the experiment reported by Lindner et al.

Lindner et al contended that each countable lesion reduces the potential number of lesions by the mean number of cells per lesion so that $Y = \frac{sfC}{L}$, where Y is the maximum number of lesions that can be formed, C is the number of epidermal cells per leaf, sf is the fraction of epidermal cells that can be infected, and L is the mean number of epidermal cells per lesion. However, it seems more reasonable to me that each countable lesion reduces the potential number of lesions, not by the mean number of epidermal cells per lesion, but only by the number of cells per lesion *that could have been infected* at the time of inoculation. The expression for Y thus becomes $sf(C\text{-}YL)$ or $\frac{sfC}{1 + sfL}$. For instance, if a leaf has 10^6 epidermal cells of which 10^2 can be infected ($C = 10^6$, $sf = 10^{-4}$, $sfC = 10^2$), and if the lesion size (L) is 10^2 epidermal cells, then the maximum number of lesions would be 99, rather than 1 as calculated with the equation of Lindner et al.

To demonstrate that it is unlikely that coincidence of infected cells limits the number of lesions in the system of Lindner et al, consider that they observe a maximum of 148 lesions per leaf with a mean area of 137 epidermal cells per lesion. Since their leaf has 1.16×10^6 epidermal cells, only a small fraction (about 2%) of the cells of the leaf are involved in lesions. It thus becomes unlikely that countable lesions are lost because several infectible cells are included in each lesion, unless the infectible cells occur in clusters.

Lindner et al estimated the value for sf in their experiment to be 1.75×10^{-2}, which would mean that 2.03×10^4 epidermal cells on a leaf can be infected. Since they observed a maximum of only 148 lesions per leaf, it follows that each lesion formed prevented 137 lesions from being formed. There are also 137 epidermal cells per lesion. It follows from their argument that all the cells involved in a lesion could have been infected at the time of inoculation. This is equivalent to saying that the leaf has 148

infectible sites, each consisting of 137 contiguous epidermal cells, a concept for which there is no independent evidence.—Department of Botany, University of California, Los Angeles.

LITERATURE CITED

1. Bald, J. G. 1937. The use of numbers of infections for comparing the concentration of plant virus suspensions. Ann. Appl. Biol. **24**: 33-55.
2. Lindner, R. C., H. C. Kirkpatrick, and T. E. Weeks. 1959. Some factors affecting the susceptibility of cucumber cotyledons to infection by tobacco mosaic virus. Phytopathology **49**: 78-88.
3. Youden, W. J., H. P. Beale, and J. D. Guthrie. 1935. Relation of virus concentration to the number of lesions produced. Contribs. Boyce Thompson Inst. **7**: 37-53.

BOOK LISTINGS

Baldys, E. ed. 1958. **Zemedelska Fytopatologie. Dil II. Choroby Polnich Plodin** (Agricultural Phytopathology. Vol. 2, Diseases of Field Crops.) State Publishing House. Prague, Czechoslovakia. 776 p. Price not stated.

This volume, written by 13 Czechoslovakian phytopathologists, deals with the physiogenic, virus, bacterial, fungus, and flowering-parasite diseases of field crops. The compilation includes discussion of a very large number of diseases on a wide variety of field crops, root crops, leguminous food crops, forage legumes, and pasture grasses.

The section on diseases of field crops includes the common grain crops, such as wheat, rye, barley, oats, corn, sorghum, millet, rice, and flax. Less common crop plants included are buckwheat, hemp, rape, mustard, hops, poppy, sunflower, safflower, and soybeans. A section on root-crop diseases includes discussions on sugar beet, chicory, and potato diseases. Two sections deal with the diseases of leguminous crops—food crops such as peas, beans, broadbeans, lentils, and chickpea; and forage crops such as clover, alfalfa, trefoil, and sweetpea. A fairly long section on the diseases of pasture grasses is included.

This compilation may be useful as a reference on diseases of less common field crops such as buckwheat, hemp, poppy, chickory, lentils, and others. Since many of the diseases discussed are not common in North America, the book should prove of interest to many plant pathologists here. This compilation is generally comprehensive, and is well illustrated with 446 figures.

Johnson, Leander F., Elroy A. Curl, John H. Bond, and Henry A. Fribourg. 1959. **Methods for Studying Soil Microflora—Plant Disease Relationships.** Burgess Publishing Company, Minneapolis. 178 p. Price $3.50.

This manual, prepared under Southern Regional Project S-26, brings together techniques for the study of soil microflora in relation to plant disease. Titles of 5 of the 15 chapters include "Taking soil samples," "Isolation of microorganisms from soil," "Growth and survival of microorganisms in soil," and "Utilization of antagonistic microorganisms for control of soil-borne plant diseases." A chapter is also devoted to "Data analysis, experimental design and statistical procedures."